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(54) Title: HEPATOCELLULAR CARCINOMA-RELATED GENES AND POLYPEPTIDES, AND METHOD FOR DETECTING HEPATOCELLULAR CARCINOMAS

(57) Abstract: Genes up-regulated in hepatocellular carcinomas and polypeptides encoded by these genes are provided. Vectors, transformants and methods for producing the recombinant polypeptides are also provided. Probes and primers of these genes and antibodies against the polypeptides are also provided. The probes, primers and antibodies can be used as reagents for detecting hepatocellular carcinomas. Methods for detecting hepatocellular carcinomas using such detection reagents are further provided. Antisense nucleotide sequences of these genes are also provided and can be used to inhibit growth of hepatocellular carcinomas.

DESCRIPTION

HEPATOCELLULAR CARCINOMA-RELATED GENES AND POLYPEPTIDES, AND METHOD FOR DETECTING HEPATOCELLULAR CARCINOMAS

Technical Field

The present invention relates to genes up-regulated in hepatocellular carcinomas, polypeptides encoded by the genes, and a method for detecting hepatocellular carcinomas.

Background Art

cDNA microarray technologies have enabled one to obtain comprehensive profiles of gene expression in normal versus malignant cells (Perou, C. M. et al., Nature. *406:* 747-752, 2000; Clark, E. A. et al., Nature. *406:* 532-535, 2000; Okabe, H. et al., Cancer Res. *61:* 2129-2137, 2001). This approach discloses the complex nature of cancer cells, and helps to improve understanding of carcinogenesis. Identification of genes that are deregulated in tumors can lead to more precise and accurate diagnosis of individual cancers, and to development of novel therapeutic targets (Golub, T. R. et al., Science 286: 531-537, 1999).

Hepatocellular carcinoma (HCC) is a leading cause of cancer deaths worldwide. In spite of recent progress in therapeutic strategies, prognosis of patients with advanced HCC remains very poor. Although molecular studies have revealed that alterations of *TP53*, *CTNNB1* and/or *AXIN1* genes can be involved in hepatocarcinogenesis (Perou, C. M. et al., Nature. *406:* 747-752, 2000; Satoh, S. et al., Nat Genet. *24:* 245-250, 2000), these changes appear to be implicated in only a fraction of HCCs. Accordingly, a ultimate gene that can be a novel diagnostic marker and/or drug target for treatment of cancers has been desired.

The present inventors previously reported that a novel gene, VANGL1, was identified by genome-wide analysis of HCCs (Yagyu, R. et al., International Journal of Oncology *20:*

1173-1178, 2002).

Disclosure of the Invention

An objective of the present invention is to provide genes up-regulated in hepatocellular carcinomas, polypeptides encoded by the genes, and a method for detecting hepatocellular carcinomas.

The present inventors have analyzed expression profiles of HCCs by means of a cDNA microarray representing 23,040 genes. These efforts have pinpointed 165 genes, including 69 ESTs, which appear to be up-regulated frequently in cancer tissues compared with corresponding non-cancerous liver cells. The inventors isolated three genes from among the transcripts whose expression was frequently elevated in HCCs. These genes encode products that shared structural features with centaurin-family proteins.

One of the three genes corresponds to an EST, Hs.44579 of a UniGene cluster, and was found to be a novel gene over-expressed at chromosomal band 1p36.13. Since an open reading frame of this gene encoded a protein approximately 60% identical to that of development and differentiation enhancing factor 2 (*DDEF2*), the inventors termed this gene development and differentiation enhancing factor-like 1 (*DDEFL1*).

Another gene up-regulated in HCCs corresponds to an EST (Hs. 122730) of a UniGene cluster. The predicted amino acid sequence shared 40% and 63% identity with *strabismus* (*Van Gogh*), which is involved in cell polarity and cell fate decisions in *Drosophila*, and *Van Gogh Like 2* (*VANGL2*). Hence, this gene was termed *Van Gogh Like 1* (*VANGL1*).

Another gene up-regulated in HCCs was found to be *LGN* (GenBank accession number U54999). *LGN* protein interacts with alpha subunit of inhibitory heterotrimeric G proteinis ($G\alpha_{i2}$).

Gene transfer of *DDEFL1* or *LGN* promoted proliferation of cells that lacked endogenous expression of either of these genes. Furthermore, reduction of *DDEFL1*, *VANGL1* or *LGN* expression by transfection of their specific anti-sense

S-oligonucleotides inhibited the growth of hepatocellular carcinoma cells.

The above findings would contribute to clarify the mechanisms of HCC and to develop new strategies for diagnosis and treatment of HCC.

The present invention specifically provides

(1) an isolated nucleic acid selected from the group consisting of:

(a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 1 or NO: 3;

(b) a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or NO: 4;

(c) a nucleic acid comprising a strand that hybridizes under high stringent conditions to a nucleotide sequence consisting of SEQ ID NO: 1 or NO: 3 or the complement thereof,

(2) an isolated polypeptide selected from the group consisting of:

(a) a polypeptide encoded by the nucleotide sequence of SEQ ID NO: 1 or NO: 3;

(b) a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or NO: 4;

(c) a polypeptide having at least 65% identity to SEQ ID NO: 2 or NO: 4,

(3) a vector carrying the nucleic acid of (1),

(4) a transformant carrying the nucleic acid of (1) or the vector of (3),

(5) a method of producing a polypeptide, the method comprising culturing the transformant of (4) in a culture, expressing the polypeptide in the transformant, and recoverying the polypeptide from the culture,

(6) an antibody that specifically binds to the polypeptide of (2),

(7) a method for detecting hepatoceullar carcinoma, the method comprising the steps of:

(a) preparing a biological sample from a subject;

(b) measuring the expression level of at least one of

polypeptides selected from the group consisting of the polypeptide of SEQ ID NO: 1, a polypeptide of SEQ ID NO: 3, and the polypeptide of SEQ ID NO: 5;

(c) comparing the expression level with that measured in a non-cancerous sample; and

(d) determining the presence or absence of the cancer in the subject,

(8) a reagent for detecting hepatocellular carcinomas, comprising a nucleic acid comprising a strand that hybridizes under high stringent conditions to a nucleotide sequence consisting of SEQ ID NO: 1, NO: 3, or NO: 5 or the complement thereof,

(9) a reagent for detecting hepatocellular carcinomas, comprising the antibody of (6), and

(10) a method for inhibiting growth of hepatocellular carcinomas, the method comprising introducing at least one of antisense oligonucleotides that hybridizes with the nucleotide sequence of SEQ ID NO: 1, NO: 3, or NO: 5 into hepatocelluar carcinomas.

The present invention will be illustrated below in more detail.

Nucleic Acids

The present invention provides genes up-regulated in hepatocellular carcinomas.

The nucleotide sequence and the amino acid sequence of *DDEFL1* are shown as SEQ ID NO: 1 and NO: 2, respectively. The complete cDNA of *DDEFL1* consisted of 4050 nucleotides, with an open reading frame of 2712 uncleotides encoding a 903-amino-acid protein (GenBank accession number AB051853). The amino acid sequence of *DDFEL1* showed 60% identity to *DDEFL2* and 46% identity to *DDEF/ASAP1*, and contained an Arf GTPase-activating protein (ArfGAP) domain and two ankyrin repeats.

DDEFL1 showed 60% identity to a member of the centaurin family, *DDEF2*, a protein that regulates re-organization of the actin cytoskeleton. This suggests that *DDEFL1* may also play a role in organization of cellular structure (Randazzo, P. A. et al., The Arf GTPase-activating protein *ASAP1* regulates the actin cytoskeleton, Proc. Natl. Acad. Sci. U S A *97:* 4011-4016, 2000). Because *DDEFL1* also conserves a PH domain and an ArfGAP motif it appears to be a new member of the centaurin family, regulating Arf small GTPase by means of GAP activity. The PH domain, observed in the majority of molecules belonging to the Dbl family of GEFs, is thought to play a crucial role in relocation of proteins by interacting with specific target molecules and/or by directly regulating catalytic domains (Jackson, T. R. et al., Trends Biochem Sci. *25:* 489-495, 2000; Cerione, R. A. and Zheng, Y., Curr. Opin. Cell. Biol. *8:* 216-222, 1996; Chardin, P. et al., Nature *384:* 481-484, 1996). Although *DDEF2* is localized in peripheral focal adhesions, the inventors found myc-tagged *DDEFL1* protein to be diffuse in cytoplasm.

Arf proteins have been implicated in important cellular processes such as vesicular membrane transport, maintenance of the integrity of ER and Golgi compartments, and regulation of the peripheral cytoskeleton (Cukierman, E. et al., Science *270:* 1999-2002, 1995). Six members of Arf family (Arf1-Arf6) and their functions have been identified so far (Moss, J. and Vaughan, M., J. Biol. Chem. *270:* 12327-12330, 1995). For example, Arf6 proteins have been implicated as regulators of the cytoskeleton to alter the morphology of focal adhesions and to block spreading of cells, and *DDEF2* displays GAP activity toward Arf1.

Over-expression of *DDEFL1* promoted growth promotion and survival of cells under low-serum conditions. This suggests that *DDEFL1* may provide a growth advantage to cancer cells in poor nutritional and hypoxic conditions. The frequent up-regulation of *DDEFL1* in HCCs underscores the importance of this gene in hepatocarcinogenesis.

The nucleotide sequence and the amino acid sequence of VANGL1 are shown as SEQ ID NO: 3 and NO: 4, respectively. The

determined cDNA sequence consisted of 1879 nucleotides containing an open reading frame of 1572 nucleotides encoding a 524-amino-acid protein (GenBank accession number AB057596).

Strabismus (*stbm*) was identified as a gene responsible for a mutant fruit fly with rough eye phenotype (Wolff T. and Rubin G.M., Development *125*:1149-1159, 1998). The gene is required to maintain polarity in the eye, legs and bristles and to decide cell fate of R3 and R4 photoreceptors in the *Drosophila*. A mouse gene homologous to *stbm*, *Ltap*, was altered in the neural tube mutant mouse Loop-tail, which is a human model of neural tube defects (NTDs) (Kibar Z et al., Nat Genet. *28*: 251-255, 2001). Hence, *VANGL1* may also play important roles in cellular polarity, cell fate decision, and/or organization of tissues. Since *VANGL1* is frequently up-regulated in HCCs and suppression of its expression significantly reduced growth or survival of cancer cells, *VANGL1* may confer prolonged survival and/or depolarized growth to cancer cells.

The nucleotide sequence and the amino acid sequence of *LGN* are shown as SEQ ID NO: 5 and NO: 6, respectively. *LGN* cDNA consists of 2336 nucleotides and encodes a 677 amino acid peptide.

LGN protein was previously reported as a protein interacting with alpha subunit of inhibitory heterotrimeric G proteins (Gαi2) (Mochizuki, N. et al., Gene *181:* 39-43, 1996). The activating mutations of Gαi2 have ever been reported in pituitary tumor and other endocrine tumors (Hermouet, S. et al., Proc. Natl. Acad. Sci. USA *88:* 10455-10459, 1991; Pace, A. M. et al., Proc. Natl. Acad. Sci. USA. *88:* 7031-7035, 1991; Lyons, J. et al, Science *249:* 655-659, 1990). However, involvement of *LGN* in tumorigenesis or carcinogenesis has not yet been reported. Colony formation assay suggested that *LGN* might have oncogenic activity. Enhanced expression of *LGN* may activate Gαi2 and mediate oncogenic signals in hepatocarcinogenesis.

The nucleic acid of the present invention includes cDNA, genomic DNA, chemically synthesized DNA, and RNA. It may be

single-stranded or double-stranded.

The "isolated nucleic acid" used herein means a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore includes, for example, (a) a DNA which has the sequence of part of a naturally occurring genomic DNA molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids of DNA molecules present in mixtures of different (i) DNA molecules, (ii) transfected cells, or (iii) cell clones; e.g., as these occur in a DNA library such as a cDNA or genomic DNA library.

In one embodiment, the nucleic acid of the present invention includes a nucleic acid comprising the nucleotide sequence of *DDEFL1* or *VANGL1*, specifically SEQ ID NO: 1 or NO: 3.

In another embodiment, the nucleic acid of the present invention includes a nucleic acid encoding a polypeptide comprising the amino acid sequence of *DDEFL1* or *VANGL1*, specifically, SEQ ID NO: 2 or NO: 4. Thus, the nucleic acid comprising arbitrary sequences based on the degeneracy of the genetic code are included.

In still another embodiment, the nucleic acid of the present invention includes a variant nucleic acid of SEQ ID NO: 1 or NO: 3. The variant includes a nucleic acid comprising a strand that hybridizes under high stringent conditions to a nucleotide sequence consisting of SEQ ID NO: 1 or NO: 3 or

the complement thereof.

The term "complement" used herein means one strand of a double-stranded nucleic acid, in which all the bases are able to form base pairs with a sequence of bases in another strand. Also, "complementary" is defined as not only those completely matching within a continuous region of at least 15 contiguous nucleotides, but also those having identity of at least 65%, preferably 70%, more preferably 80%, still more preferably 90%, and most preferably 95% or higher within that region.

As used herein, "percent identity" of two nucleic acids is determined using the algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 87: 2264-2268, 1990) modified as in Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90:5873-5877, 1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (J. Mol. Biol. 215:403-410, 1990). BLAST nucleotide searches are performed with the NBLAST program, score = 100, wordlength = 12. Homology search of protein can readily be performed, for example, in DNA Databank of JAPAN (DDBJ), by using the FASTA program, BLAST program, etc. BLAST protein searches are performed with the XBLAST program, score = 50, wordlength = 3. Where gaps exist between two sequences, Gapped BLAST is utilized as described in Altsuchl et al. (Nucleic Acids Res. 25: 3389-3402, 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g, XBLAST and NBLAST) are used.

Preferably, the variant includes a nucleotide sequence that is at least 65% identical to the nucleotide sequence shown in SEQ ID NO: 1 or NO: 3. More preferably, the variant is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more, identical to the nucleotide sequence shown in SEQ ID NO: 1 or NO: 3. In the case of a variant which is longer than or equivalent in length to the reference sequence, *e.g.*, SEQ ID NO: 1 or NO: 3, the comparison is made with the full length of the reference sequence. Where the variant is shorter than the reference sequence, *e.g.*, shorter than SEQ ID NO: 1 or NO: 3, the comparison is made to segment of the reference sequence

of the same length (excluding any loop required by the homology calculation).

The stringency of hybridization is defined as equilibrium hybridization under the following conditions: 42℃, 2 x SSC, 0.1% SDS (low stringency); 50℃, 2 x SSC, 0.1% SDS (medium stringency); and 65℃, 2 x SSC, 0,1% SDS (high stringency). If washings are necessary to achieve equilibrium, the washings are performed with the hybridization solution for the particular stringency desired. In general, the higher the temperature, the higher is the homology between two strands hybridizing at equilibrium.

There is no restriction on length of the nucleic acid of the present invention, but it preferably comprises at least 15, 20, 30, 40, 50, 100, 150, 200, 300, 400, 500, 1000, 1500, 2000, 2500, or 3000 nucleotides.

The nucleic acid of the present invention includes polynucleotides used as probes or primers specifically hybridizing with the nucleotide sequence of SEQ ID NO: 1 or NO: 3 or its complement. The term "specifically hybridizing" means that hybridizing under a normal hybridization condition, preferably a stringent condition with the nucleotide sequence of SEQ ID NO: 1 or NO: 3, but not crosshybridizing with DNAs encoding other polypeptides.

The primers and probes comprise at least 15 continuous nucleotides within the nucleotide sequence of SEQ ID NO: 1 or 3 or complementary to the sequence. In general, the primers comprises 15 to 100 nucleotides, and preferably 15 to 35 nucleotides, and the probes comprise at least 15 nucleotides, preferably at least 30 nucleotides, containing at least a portion or the whole sequence of SEQ ID NO: 1 or NO: 3. The primers can be used for amplification of the nucleic acid encoding the polypeptide of the present invention and the probes can be used for the isolation or detection of the nucleic acid encoding the polypeptide of the present invention. The primers and probes of the present invention can be prepared, for example, by a commercially available oligonucleotide synthesizing machine.

The probes can be also prepared as double-stranded DNA fragments which are obtained by restriction enzyme treatments and the like.

The nucleic acid of the present invention includes an antisense oligonucleotide that hybridizes with any site within the nucleotide sequence of SEQ ID NO: 1 or 3. The term "antisense oligonucleotides" as used herein means, not only those in which the entire nucleotides corresponding to those constituting a specified region of a DNA or mRNA are complementary, but also those having a mismatch of one or more nucleotides, as long as DNA or mRNA and an oligonucleotide can specifically hybridize with the nucleotide sequence of SEQ ID NO: 1 or NO: 3.

The antisense oligonucleotide is preferably that against at least 15 continuous nucleotides in the nucleotide sequence of SEQ ID NO: 1 or NO: 3. The above-mentioned antisense oligonucleotide, which contains an initiation codon in the above-mentioned at least 15 continuous nucleotides, is even more preferred.

The antisense oligonucleotides of the present invention includes analogs containing lower alkyl phosphonate (e.g., methyl-phosphonate or ethyl-phosphonate), phosphothioate, and phosphoamidate.

The antisense oligonucleotide of the present invention, acts upon cells producing the polypeptide of the invention by binding to the DNA or mRNA encoding the polypeptide and inhibits its transcription or translation, promotes the degradation of the mRNA, inhibiting the expression of the polypeptide of the invention.

The nucleic acid of the present invention can be prepared as follows. cDNA encoding the polypeptide of the present invention can be prepared, for example, by preparing a primer based on nucleotide information (for example, SEQ ID NO: 1 or NO: 3) of DNA encoding the polypeptide of the present invention and performing plaque PCR (Affara NA et al. (1994) Genomics 22, 205-210). Genomic DNA can be prepared, for example, by the method using commercially available "Qiagen genomic DNA

kits" (Qiagen, Hilden, Germany). The nucleotide sequence of the DNA acquired can be decided by ordinary methods in the art by using, for example, the commercially available "dye terminator sequencing kit" (Applied Biosystems). The nucleic acid of the present invention, as stated later, can be utilized for the production of a recombinant protein and detection of hepatocellular carcinoma.

Vectors, Transformants, and Production of Recombinant Polypeptide

The present invention also features a vector into which the nucleic acid of the present invention has been inserted.

The vector of the present invention includes a vector for preparing the recombinant polypeptide of the present invention. Any vector can be used as long as it enables expression of the polypeptide of the present invention.

Examples of the expression vector include bacterial (e.g. *Escherichia coli*) expression vectors, yeast expression vectors, insect expression vectors, and mammalian expression vectors. In the present invention, mammalian expression vectors such as pcDNA3.1-myc/His or pcDNA 3.1 vector (Invitrogen) can be used. Insertion of the nucleic acid of the present invention into a vector can be done using ordinary methods in the art.

The vector of the present invention also includes a vector for expressing the polypeptide of the present invention *in vivo* (especially for gene therapy). Various viral vectors and non-viral vectors can be used as long as they enable expression of the polypeptide of the present invention *in vivo*. Examples of viral vectors are adenovirus vectors, retrovirus vectors, etc. Cationic liposomes can be given as examples of non-viral vectors.

The present invention also provides a transformant carrying, in an expressible manner, the nucleic acid of the present invention. The transformant of the present invention includes, those carrying the above-mentioned expression vector

into which nucleic acid of the present invention has been inserted, and those having host genomes into which the nucleic acid of the present invention has been integrated. The nucleic acid of the invention is retained in the transformant in any form as long as the transformant can express the nucleic acid.

There is no particular restriction as to the cells into which the vector is inserted as long as the vector can function in the cells to express the nucleic acid of the present invention. For example, *E. coli*, yeast, mammalian cells and insect cells can be used as hosts. Preferably, mammalian cells such as COS7 cells and NIH3T3 cells. Introduction of a vector into a cell can be done using known methods such as electroporation and calcium phosphate method.

Common methods applied in the art may be used to isolate and purify said recombinant polypeptide from the transformant. For example, after collecting the transformant and obtaining the extracts, the objective polypeptide can be purified and prepared by, ion exchange chromatography, reverse phase chromatography, gel filtration, or affinity chromatography where an antibody against the polypeptide of the present invention has been immobilized in the column, or by combining several of these columns.

Also when the polypeptide of the present invention is expressed within host cells (for example, animal cells, *E. coli*) as a fusion protein with glutathione-S-transferase protein or as a recombinant polypeptide supplemented with multiple histidines, the expressed recombinant polypeptide can be purified using a glutathione column or nickel column. After purifying the fusion protein, it is also possible to exclude regions other than the objective polypeptide by cutting with thrombin or factor-Xa as required.

Polypeptides

The present invention provides isolated polypeptides encoded by *DDEFL1* or *VANGL1* (e.g. SEQ ID NO: 1 or NO: 3). In

specific embodiments, the polypeptides of the present invention includes a polypeptide encoded by the nucleotide sequence of SEQ ID NO: 1 or NO: 3 and a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or NO: 4.

The "isolated polypeptide" used herein means a polypeptide that is substantially pure and free from other biological macromolecules. The substantially pure polypeptide is at least 75% (e.g., at least 80, 85, 95, or 99%) pure by dry weight. Purity can be measured by any appropriate standard method, for example by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

The polypeptide of the present invention includes variants of SEQ ID NO: 2 or NO: 4 as long as the variants are at least 65% identical to SEQ ID NO: 2 or NO: 4. The variants may be a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or NO: 4 in which one or more amino acids have been substituted, deleted, added, and/or inserted. The variants may also be a polypeptide encoded by a nucleic acid comprising a strand that hybridizes under high stringent conditions to a nucleotide sequence consisting of SEQ ID NO: 1 or NO: 3.

Polypeptides having amino acid sequences modified by deleting, adding and/or replacing one or more amino acid residues of a certain amino acid sequence, have been known to retain the original biological activity (Mark, D. F. et al., Proc. Natl. Acad. Sci. USA (1984) 81, 5662-5666, Zoller, M. J. & Smith, M., Nucleic Acids Research (1982) 10, 6487-6500, Wang, A. et al., Science 224, 1431-1433, Dalbadie-McFarland, G. et al., Proc. Natl. Acad. Sci. USA (1982) 79, 6409-6413).

The number of amino acids that are mutated by substitution, deletion, addition, and/or insertion is not particularly restricted. Normally, it is 10% or less, preferably 5% or less, and more preferably 1% or less of the total amino acid residues.

As for the amino acid residue to be mutated, it is preferable to be mutated into a different amino acid in which the properties of the amino acid side-chain are conserved. Examples of properties of amino acid side chains are, hydrophobic amino

acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and amino acids comprising the following side chains: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain (H, F, Y, W) (The parenthetic letters indicate the one-letter codes of amino acids). A "conservative amino acid substitution" is a replacement of one amino acid belonging to one of the above groups with another amino acid in the same group.

A deletion variant includes a fragment of the amino acid sequence of SEQ ID NO: 1 or NO: 3. The fragment is a polypeptide having an amino acid sequence which is partly, but not entirely, identical to the above polypeptides of this invention. The polypeptide fragments of this invention usually consist of 8 amino acid residues or more, and preferably 12 amino acid residues or more (for example, 15 amino acid residues or more). Examples of preferred fragments include truncation polypeptides, having amino acid sequences lacking a series of amino acid residues including either the amino terminus or carboxyl terminus, or two series of amino acid residues, one including the amino terminus and the other including the carboxyl terminus. Furthermore, fragments featured by structural or functional characteristics are also preferable, which include those having α-helix and α-helix forming regions, β-sheet and β-sheet forming regions, turn and turn forming regions, coil and coil forming regions, hydrophilic regions, hydrophobic regions, α-amphipathic regions, β-amphipathic regions, variable regions, surface forming regions, substrate-binding regions, and high antigenicity index region. Biologically active fragments are also preferred. Biologically active fragments mediate the activities of the polypeptides of this invention, which fragments include those having similar or improved activities, or reduced undesirable activities. For example, fragments having the activity to transduce signals into cells *via* binding

of a ligand, and furthermore, fragments having antigenicity or immunogenicity in animals, especially humans are included. These polypeptide fragments preferably retain the antigenicity of the polypeptides of this invention.

Further, an addition variant includes a fusion protein of the polypeptide of the present invention and another peptide or polypeptide. Fusion proteins can be made by techniques well known to a person skilled in the art, such as by linking the DNA encoding the polypeptide of the invention with DNA encoding other peptides or polypeptides, so as the frames match, inserting this into an expression vector and expressing it in a host. There is no restriction as to the peptides or polypeptides fused to the polypeptide of the present invention.

Known peptides, for example, FLAG (Hopp, T.P. et al., Biotechnology (1988) 6, 1204-1210), 6xHis containing six His (histidine) residues, 10xHis, Influenza agglutinin (HA), human c-myc fragment, VSP-GP fragment, p18HIV fragment, T7-tag, HSV-tag, E-tag, SV40T antigen fragment, lck tag, α-tubulin fragment, B-tag, Protein C fragment, and such, can be used as peptides that are fused to the polypeptide of the present invention. Examples of polypeptides that are fused to polypeptide of the invention are, GST (glutathione-S-transferase), Influenza agglutinin (HA), immunoglobulin constant region, β-galactosidase, MBP (maltose-binding protein), and such.

Fusion proteins can be prepared by fusing commercially available DNA encoding these peptides or polypeptides with the DNA encoding the polypeptide of the present invention and expressing the fused DNA prepared.

The variant polypeptide is preferably at least 65% identical to the amino acid sequence shown in SEQ ID NO: 2 or NO: 4. More specifically, the modified polypeptide is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more, identical to the amino acid sequence shown in SEQ ID NO: 2 or NO: 4. In the case of a modified polypeptide which is longer than or equivalent in length to the reference sequence, *e.g.*,

SEQ ID NO: 2 or NO: 4, the comparison is made with the full length of the reference sequence. Where the modified polypeptide is shorter than the reference sequence, *e.g.*, shorter than SEQ ID NO: 2 or NO: 4, the comparison is made to segment of the reference sequence of the same length.

As used herein, "percent identity" of two amino acid sequences is determined in the same manner as described above for the nucleic acids.

The polypeptide of the present invention can be prepared by methods known to one skilled in the art, as a natural polypeptide or a recombinant polypeptide made using genetic engineering techniques as described above. For example, a natural polypeptide can be obtained by preparing a column coupled with an antibody obtained by immunizing a small animal with the recombinant polypeptide, and performing affinity chromatography for extracts of liver tissues or cells expressing high levels of the polypeptide of the present invention. A recombinant polypeptide can be prepared by inserting DNA encoding the polypeptide of the present invention (for example, DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3) into a suitable expression vector, introducing the vector into a host cell, allowing the resulting transformant to express the polypeptide, and recovering the expressed polypeptide.

The variant polypeptide can be prepared, for example, by inserting a mutation into the amino acid sequence of SEQ ID NO: 1 or NO: 3 by a known method such as the PCR-mediated, site-directed-mutation-induction system (GIBCO-BRL, Gaithersburg, Maryland), oligonucleotide-mediated, sight-directed-mutagenesis (Kramer, W. and Fritz, HJ (1987) Methods in Enzymol. 154:350-367).

Antibodies

The present invention also features an antibody that specifically binds to the polypeptide of the present invention. There is no particular restriction as to the form of the antibody

of the present invention and include polyclonal antibodies and monoclonal antibodies. The antiserum obtained by immunizing animals such as rabbits with the polypeptide of the present invention, polyclonal and monoclonal antibodies of all classes, humanized antibodies made by genetic engineering, human antibodies, are also included.

Polyclonal antibodies can be made by, obtaining the serum of small animals such as rabbits immunized with the polypeptide of the present invention, attaining a fraction recognizing only the polypeptide of the invention by an affinity column coupled with the polypeptide of the present invention, and purifying immunoglobulin G or M from this fraction by a protein G or protein A column.

Monoclonal antibodies can be made by immunizing small animals such as mice with the polypeptide of the present invention, excising the spleen from the animal, homogenizing the organ into cells, fusing the cells with mouse myeloma cells using a reagent such as polyethylene glycol, selecting clones that produce antibodies against the polypeptide of the invention from the fused cells (hybridomas), transplanting the obtained hybridomas into the abdominal cavity of a mouse, and extracting ascites. The obtained monoclonal antibodies can be purified by, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE ion exchange chromatography, or an affinity column to which the polypeptide of the present invention is coupled. The antibody of the invention can be used for purifying and detecting the polypeptide of the invention. In particular, it can be used for detecting hepatocellular carcinoma.

The human antibodies or humanized antibodies can be prepared by methods commonly known to one skilled in the art. For example, human antibodies can be made by, immunizing a mouse whose immune system has been changed to that of humans, with the polypeptide of the present invention. Also, humanized antibodies can be prepared by, for example, cloning the antibody gene from monoclonal antibody producing cells and using the

CDR graft method which transplants the antigen-recognition site of the gene into a known human antibody.

Detection Methods

The present invention further provides a method of detecting hepatocellular carcinoma using the *DDEFL1*, *VANGL1*, or *LGN* polypeptide as a marker.

The detection can be performed by measuring an expression level of at least one of *DDEFL1*, *VANGL1*, and *LGN* polypeptides in a biological sample from a subject, comparing the expression level with that in a non-cancerous sample, and determining the presence or absence of the cancer in a subject.

A biological sample used herein include any liver tissues or cells obtained from a subject who is in need of detection of hepatocellular carcinoma. In particular, liver biopsy specimen can be used. The biological sample also includes an mRNA, cRNA or cDNA sample prepared from liver tissues or cells. mRNA and cDNA samples can be prepared by a conventional method. cRNA refers to RNA transcribed from a template cDNA with RNA polymerase. cRNA can be synthesized from T7 promoter-attached cDNA as a template by using T7 RNA polymerase. A commercially available cRNA transcription kit for DNA chip-based expression profiling can be used.

In specific embodiments, the expression level of *DDEFL1*, *VANGL1* or *LGN* polypeptide can be measured in the RNA, cDNA, or polypeptide level.

The mRNA expression level can be measured by, for example, a Northern blotting method using a probe that hybridizes with the nucleotide sequence of *DDEFL1*, *VANGL1*, or *LGN*, an RT-PCR method using a primer that hybridizes with the nucleotide sequence of *DDEFL1*, *VANGL1*, or *LGN*, and such.

The probes or primers used in the detection method of the present invention include a nucleic acid specifically hybridizing with the nucleotide sequence of SEQ ID NO: 1, NO: 3, or NO: 5, or its complement. The term "specifically

hybridizing" means that hybridizing under a normal hybridization condition, preferably a stringent condition with the nucleotide sequence of SEQ ID NO: 1, NO: 3, or NO: 5, but not crosshybridizing with DNAs encoding other polypeptides.

The primers and probes comprise at least 15 continuous nucleotides within the nucleotide sequence of SEQ ID NO: 1, NO: 3, or NO: 5 or complementary to the sequence. In general, the primers comprises 15 to 100 nucleotides, and preferably 15 to 35 nucleotides, and the probes comprise at least 15 nucleotides, preferably at least 30 nucleotides, containing at least a portion or the whole sequence of SEQ ID NO: 1, NO: 3, or NO: 5. The primers and probes can be prepared, for example, by a commercially available oligonucleotide synthesizing machine. The probes can be also prepared as double-stranded DNA fragments which are obtained by restriction enzyme treatments and the like.

The cDNA expression level can be measured by, for example, a method utilizing a DNA array (Masami Muramatsu and Masashi Yamamoto, New Genetic Engineering Handbook pp. 280-284, YODOSHA Co., LTD.). Specifically, first, a cDNA sample prepared from a subject and a solid support, on which polynucleotide probes hybridizing with the nucleotide sequence of DDEFL1, VANGL1, or LGN are fixed, are provided. As the probes, those as described above can be used. Plural kinds of probes can be fixed on the solid support in order to detect plural kinds of target polynucleotides. The cDNA sample is labeled for detection according to needs. The label is not specifically limited so long as it can be detected, and includes, for example, fluorescent labels, radioactive labels, and so on. The labeling can be carried out by conventional methods (L. Luo et al., "Gene expression profiles of laser-captured adjacent neuronal subtypes", Nat. Med. (1999) pp. 117-122).

The cDNA sample is then contacted with the probes on the solid support to allow the cDNA sample to hybridize with the probes. Although the reaction solution and the reaction condition for hybridization varies depending on various factors,

such as the length of the probe, they can be determined according to usual methods well known to those skilled in the art.

The intensity of hybridization between the cDNA sample and the probes on the solid support is measured depending on the kind of the label of the cDNA sample. For example, a fluorescent label can be detected by reading out the fluorescent signal with a scanner.

The hybridization intensity of the test cDNA sample and the control cDNA sample (e.g. cDNA from non-cancerous tissues or cells) can be measured simultaneously in one measurement by labeling them with different fluorescent labels. For example, one of the above-mentioned cDNA samples can be labeled with Cy5, and the other with Cy3. The intensity of Cy5 and Cy3 fluorescent signals show the expression level of the respective cDNA samples (Duggan et al., Nat. Genet. 21:10-14, 1999).

In this method, cRNA can be measured in place of cDNA.

Furthermore, the polypeptide expression level can be measured using an antibody against *DDEFL1*, *VANGL1*, or *LGN* polypeptide by, for example, SDS polyacrylamide electrophoresis, Western blotting, dot-blotting, immunoassay such as immunoprecipitation, fluoroimmunoassay, radioimmunoassay, enzyme immunoassay (e.g. enzyme-linked immunosorbent assay (ELISA)), and immunohistochemical staining, etc.

In specific embodiments, a biological sample is contacted with an antibody against *DDEFL1*, *VANGL1*, or *LGN* polypeptide immobilized on a solid support, the antibody-antigen complex on the solid support is contacted with a second antibody labeled with a detectable label, and the label is detected by an appropriate method.

The antibody used in the detection method of the present invention includes any antibody that binds to the *DDEFL1*, *VANGL1*, or *LGN* polypeptide, specifically the polypeptide with the amino acid sequence of SEQ ID NO: 2, NO: 4, or NO: 6, including antiserum obtained by immunizing animals such as rabbits with the *DDEFL1*, *VANGL1*, or *LGN* polypeptide, polyclonal and monoclonal antibodies of all classes, humanized antibodies made by genetic

engineering, and human antibodies. These antibodies can be prepared as described above.

The expression level measured as described above is compared with that measured in a non-cancerous sample to determine the presence or absence of hematocellular carcinoma in the subject. When the expression level measured in the sample from the subject is higher than that measured in the non-cancerous sample, the subject is judged to have the cancer or the risk of the cancer. On the other hand, the expression level in the subject sample is not higher compared with that in the non-cancerous sample, then, the subject is judged to be free from the cancer. Specifically, whether the expression level in the subject sample is higher than that in the non-cancerous sample, can be determined based on the relative expression ratio (subject sample/non-cancerous sample); the expression level is judged as being higher when the relative expression ratio is more than 2.0.

Detection Reagents

The present invention provides detection reagents for hepatocellular carcinomas.

In one embodiment, the detection reagent of the present invention comprises a polynucleotide having at least 15 nucleotides which hybridizes with *DDEFL1*, *VANGL1* or *LGN*, specifically SEQ ID NO: 1, NO: 3, or NO: 5. The polynucleotide can be used in the above-mentioned detection method of the present invention as a probe or a primer. When used as a probe, the polynucleotides contained in the detection reagent of the present invention can be labeled. The method of labeling includes, for example, a labeling method using T4 polynucleotide kinase to phosphorylate the 5'-terminus of the polynucleotide with ^{32}P; and a method of introducing substrate bases, which are labeled with isotopes such as ^{32}P, fluorescent dyes, biotin, and so on using random hexamer oligonucleotides and such as primers and DNA polymerase such as Klenow enzyme (the random

prime method, etc.).

In another embodiment, the detection reagent of the present invention comprises an antibody that binds to the *DDEFL1*, *VANGL1*, or *LGN* polypeptide, specifically the polypeptide having the amino acid sequence of SEQ ID NO: 2, NO: 4, or NO: 6. The antibodies are used to detect the polypeptides of the present invention in the above-mentioned detection method of the present invention. The antibodies may be labeled according to the diction method. Furthermore, the antibodies may be immobilized on a solid support.

The detection reagent of the present invention may further comprise a medium or additive, including sterilized water, physiological saline, vegetable oils, surfactants, lipids, solubilizers, buffers, protein stabilizers (such as bovine serum albumin and gelatin), preservatives, and such, as long as it does not affect the reactions used in the detection method of the present invention.

Methods for Inhibiting Growth of Hematocellular Carcinomas

The present invention further provides a method for inhibiting growth of hepatocellular carcinomas. In specific embodiments, this method can be performed by introducing an antisense oligonucleotide of *DDEFL1*, *VANGL1*, or *LGN* into the target cells.

The antisense oligonucleotide used in this method hybridizes with any site within the nucleotide sequence of SEQ ID NO: 1, NO: 3, or NO: 5. The antisense oligonucleotides include not only those in which the entire nucleotides corresponding to those constituting a specified region of a DNA or mRNA are complementary, but also those having a mismatch of one or more nucleotides, as long as DNA or mRNA and an oligonucleotide can specifically hybridize with the nucleotide sequence of SEQ ID NO: 1, NO: 3, or NO: 5.

The antisense oligonucleotide is preferably that against at least 15 continuous nucleotides in the nucleotide sequence

of SEQ ID NO: 1, NO: 3, or NO: 5. The above-mentioned antisense oligonucleotide, which contains an initiation codon in the above-mentioned at least 15 continuous nucleotides, is even more preferred.

The antisense oligonucleotides includes analogs containing lower alkyl phosphonate (e.g., methyl-phosphonate or ethyl-phosphonate), phosphothioate, and phosphoamidate.

Herein, the target cells may be mammalian cells, preferably human cells.

The introduction method may be *in vitro*, *in vivo*, or *ex vivo* transfer method. In one embodiment, the antisense oligonucleotides can be introduced into the target cells by a conventional transfection method. Alternatively, the introduction can be made by conventional gene transfer technique using a vector carrying the antisense oligonucleotide, such as adenovirus vectors, retrovirus vectors, or cationic liposomes.

Any patents, patent applications, and publications cited herein are incorporated by reference.

Brief Description of Drawings

Figure 1a-1b show expression of a gene termed B9362 in HCCs. Fig. 1a shows relative expression ratio (cancer/non-cancer) of B9362 in primary 20 HCCs examined by cDNA microarray. Fig. 1b presents photographs showing expression of B9362 analyzed by semi-quantitative RT-PCR using additional 11 HCC cases. Expression of *GAPDH* served as an internal control.

Figure 2a-2d show the results of identification of *DDEFL1*. Fig. 2a is a photograph showing the results of Northern blot analysis of *DDEFL1* in various human tissues. Fig. 2b shows the structure of *DDEFL1*. Fig. 2c shows similarity between the expected *DDEFL1* protein and members of ArfGAP family. Fig. 2d shows identity between the amino acid sequence of the ArfGAP

motif in *DDEFL1* and that in *DDEF2*. The arrows indicate a $CXXCX_{16}CXXC$ motif, representing a zinc finger structure essential to GAP activity.

Figure 3a-3b show subcellular localization of *DDEFL1*. Fig. 3a is a photograph showing the results of Western blot analysis, indicating that cMyc-tagged *DDEFL1* protein was expressed in COS7 cells transfected with pcDNA-*DDEFL1*-myc plasmid. Fig. 3b presents photographs showing immunocytochemistry of the cells, suggesting that cMyc-tagged *DDEFL1* protein localized in the cytoplasm.

Figure 4a-4d show growth-promoting effect of *DDEFL1*. Fig. 4a presents photographs showing the results of colony formation assays, indicating that *DDEFL1* promotes cell growth in NIH3T3, SNU423, and Alexander cells. Fig. 4b presents photographs showing stable expression of exogeneous *DDEFL1* by NIH3T3-*DDEFL1* cells. Fig. 4c is a graph showing growth of NIH3T3-*DDEFL1* cells stably expressing exogeneous *DDEFL1* in culture media containing 10% FBS. Fig. 4d is a graph showing growth of NIH3T3-*DDEFL1* cells in culture media containing 0.1% FBS ($P<0.01$).

Figure 5a-5b show growth suppression by antisense S-oligonucleotides designated to suppress *DDEFL1* in SNU475 cells. Fig. 5a shows designation of antisense S-oligonucleotides and photographs showing reduced expression of *DDEFL1* by the transfection of AS1 or AS5 antisense S-oligonucleotides. Fig. 5b presents photographs showing that AS1 and AS5 suppressed growth of SNU475 cells.

Figure 6a-6b show expression of *VANGL1* in HCCs. Fig. 6a shows relative expression ratios (cancer/non-cancer) of *VANGL1* in primary 20 HCCs examined by cDNA microarray. Fig. 6b presents photographs showing expression of *D3244* analyzed by semi-quantitative RT-PCR using additional 10 HCC cases. T, tumor tissue; N, normal tissue. Expression of *GAPDH* served as an internal control.

Figure 7a and 7B show the results of identification of *VANGL1*. Fig. 7a is a photograph showing the results of multiple-tissue Northern blot analysis of *VANGL1* in various

human tissues. Fig. 7b shows predicted protein structure of *VANGL1*.

Figure 8a and 8b show subcellular localization of *VANGL1*. Fig. 8a presents photographs of SNU475 cells transfected with pcDNA3.1-myc/His-*VANGL1* stained with mouse anti-myc monoclonal antibody and visualized by Rhodamine conjugated secondary anti-mouse IgG antibody. Nuclei were counter-stained with DAPI. Fig. 8b presents photographs of mock cells similarly stained and visualized.

Figure 9a-9d show growth suppressive effect of antisense S-oligonucleotide designated to suppress *VANGL1*. Fig. 9a presents photographs showing expression of *VANGL1* in SNU475 cells treated with either control or antisense oligonucleotide for 12 hours. Fig. 9b is a photograph showing that S-oligonucleotide suppressed growth of SNU423 cells. Fig. 9c is a graph showing the results of analysis of cell viability by MTT assay. Fig. 9d shows the results of fluorescence activated cell sorting (FACS) analysis of cells treated with sense or antisense oligonucleotide.

Figure 10a and 10b show *LGN* gene expression of HCCs compared with their corresponding non-cancerous liver tissues. Fig. 10a shows relative expression ratios (cancer/non-cancer) of *LGN* in primary 20 HCCs studied by cDNA microarray. Fig. 10b presents photographs showing expression of *LGN* analyzed by semi-quantitative RT-PCR using additional ten HCCs. Expression of *GAPDH* served as an internal control. T, tumor tissue; N, normal tissue.

Figure 11 shows genomic structure of *LGN*.

Figure 12a-12c show subcellular localization of LGN. Fig. 12a is a photograph of COS7 cells transfected with pcDNA3.1-myc/His-*LGN*, in which nuclei was counter-stained with DAPI. Fig. 12b is a photograph of COS7 cells transfected with pcDNA3.1-myc/His-*LGN*, which were stained with mouse anti c-myc antibody and visualized by Rhodamine conjugated secondary anti-mouse IgG antibody. Fig. 12c is a merge of a and b.

Figure 13a and 13b show growth-promoting effect of *LGN*.

Fig. 13a presents photographs showing the results of colony formation assays, indicating that *LGN* promotes cell growth in NIH3T3, SNU423, Alexander, and SNU475 cells. Fig. 13b is a graph showing growth of NIH3T3-*LGN* cells stably expressing exogeneous *LGN* was higher than that of mock (NIH3T3-*LacZ*) cells in culture media containing 10% FBS.

Figure 14a and 14b show growth suppression by antisense S-oligonucleotide designated to suppress *LGN* expression in human hepatoma SNU423 cells. Fig. 14a presents photographs showing reduced expression of *LGN* by the transfection of antisense S-oligonucleotide, antisense 3. Fig. 4b is a photograph showing that antisense 3 suppressed growth of SNU423 cells.

Best Mode for Carrying out the Invention

The present invention will be illustrated with reference to the following examples, but is not construed as being limited thereto.

Example 1

1-1. Identification of *DDEFL1* commonly up-regulated in human hepatocellular carcinomas

By means of a genome-wide cDNA microarray containing 23040 genes, expression profiles of 20 hepatocellular carcinomas (HCC) were compared with their corresponding non-cancerous liver tissues. All HCC tissues and corresponding non-cancerous tissues were obtained with informed consent from surgical specimens of patients who underwent hepatectomy. A gene with an in-house accession number of *B9362* corresponding to an EST, Hs.44579 of a UniGene cluster, was found to be over-expressed in a range between 1.57 and 5.83 (Fig. 1a). Its up-regulated expression (Cy3:Cy5 intensity ratio, >2.0) was observed in 11 of the 12 HCCs that passed through the cutoff filter (both Cy3 and Cy5 signals greater than 25,000). Since an open reading frame of this gene encoded a protein approximately 60% identical to that of development and differentiation enhancing factor

2 (*DDEF2*), this gene was termed development and differentiation enhancing factor-like 1 (*DDEFL1*). To clarify the results of the cDNA microarray, expression of this transcript was examined in an additional 11 HCCs by semi-quantitative RT-PCR. Expression of *GAPDH* served as an internal control. RT-PCR was performed as follows. Total RNA was extracted with a Qiagen RNeasy kit (Qiagen) or Trizol reagent (Life Technologies, Inc.) according to the manufacturers' protocols. Ten-microgram aliquots of total RNA were reversely transcribed for single-stranded cDNAs using poly dT_{12-18} primer (Amersham Pharmacia Biotech) with Superscript II reverse transcriptase (Life Technologies). Single-stranded cDNA preparation was diluted for subsequent PCR amplification by standard RT-PCR experiments carried out in 20-μl volumes of PCR buffer (TAKARA). Amplification proceeded for 4 min at 94°C for denaturing, followed by 20 (for *GAPDH*) or 33 (for *DDEFL1*) cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 45 s, in the GeneAmp PCR system 9700 (Perkin-Elmer, Foster City, CA). Primer sequences were; for *GAPDH*: forward, 5'-ACAACAGCCTCAAGATCATCAG (SEQ ID NO: 7) and reverse, 5'-GGTCCACCACTGACACGTTG (SEQ ID NO: 8); for *DDEFL1*: forward, 5'-AGCTGAGACATTTGTTCTCTTG (SEQ ID NO: 9) and reverse: 5'-TATAAACCAGCTGAGTCCAGAG (SEQ ID NO: 10). The results confirmed increased expression of *DDEFL1* in nine of these tumors (Fig. 1b).

1-2. Isolation and structure of a novel gene *DDEFL1*

Expression of *DDEFL1* was analyzed by multiple-tissue northern-blot analysis using a PCR product of *DDEFL1* as a probe. Human multiple-tissue blots (Clontech, Palo Alto, CA) were hybridized with a ^{32}P-labeled *DDEFL1* cDNA. Pre-hybridization, hybridization and washing were performed according to the supplier's recommendations. The blots were autoradiographed with intensifying screens at -80°C for 72 h. The results revealed a 4-kb transcript that was expressed in lung, liver, small intestine, placenta and peripheral blood leukocyte (Fig. 2a).

Since *B9362* was smaller than that detected on the Northern blot, 5'RACE experiments was carried out to determine the entire

coding sequence of the gene. 5' RACE experiments were carried out using a Marathon cDNA amplification kit (Clontech, Palo Alto, CA) according to the manufacturer's instructions. For the amplification of the 5' part of *DDEFL1* cDNAs, gene-specific reverse primers (5'-CTCACTTGGCACGTCAGCAGGG (SEQ ID NO: 11)) and the AP-1 primer supplied in the kit were used. The cDNA template was synthesized from human liver mRNA. The PCR products were cloned using a TA cloning kit (Invitrogen) and their sequences were determined with an ABI PRISM 3700 DNA sequencer (Applied Biosystems).

The complete cDNA consisted of 4050 nucleotides, with an open reading frame of 2712 nucleotides encoding a 903-amino-acid protein (GenBank accession number AB051853). The first ATG was flanked by a sequence (CCCGCCATGC (SEQ ID NO: 12)) that agreed with the consensus sequence for initiation of translation in eukaryotes, with an in-frame stop codon upstream. The BLAST program to search for homologies in the NCBI (the National Center for Biotechnology Information) database, identified a genomic sequence with GenBank accession number AL357134, which had been assigned to chromosomal band 1p36.12. Comparison of the cDNA and genomic sequences disclosed that *DDEFL1* consisted of 25 exons (Fig. 2b).

A search for protein motifs with the Simple Modular Architecture Research Tool (SMART) revealed that the predicted protein contained two coiled-coil regions (codons 141-172 and 241-278), a PH (Pleckstrin homology) motif (codons 303-396), a motif of ArfGAP (GTPase-activating protein for Arf) (codons 426-551) and two ankyrin repeats (codons 585-617 and 621-653). This structure was similar to centaurin beta 1 and centaurin beta 2 (Fig. 2c). In particular, *DDEFL1* shared features of centaurin-family proteins such as a PH domain, a target of phosphatidylinositol 3,4,5-trisphosphate, and a motif of ArfGAP. The amino acid sequence of the ArfGAP motif of DDEFL1 was 67.8% identical to that of *DDEF2* (Fig. 2d). Notably, the $CXXCX_{16}CXXC$ motif, representing a zinc finger structure essential to GAP activity, was completely preserved.

1-3. Subcellular localization of *DDEFL1*

The coding sequence of *DDEFL1* was cloned into the pcDNA3.1-myc/His vector (Invitrogen). The resulting plasmid expressing myc-tagged *DDEFL1* protein (pDNA-myc/His-*DDEFL1*) was transiently transfected into COS7 cells (American Type Culture Collection (ATCC)). The expected myc-tagged protein was detected by immunoblotting (Western blotting) as follows. Cells transfected with pcDNA3.1-myc/His-*DDEFL1* were washed twice with PBS and harvested in lysis buffer (150 mM NaCl, 1% Triton X-100, 50 mM Tris-HCl pH 7.4, 1mM DTT, and 1X complete Protease Inhibitor Cocktail (Boehringer)). After the cells were homogenized and centrifuged at 10,000x*g* for 30 min, the supernatant was standardized for protein concentration by the Bradford assay (Bio-Rad). Proteins were separated by 10% SDS-PAGE and immunoblotted with mouse anti-myc antibody. HRP-conjugated goat anti-mouse IgG (Amersham) served as the secondary antibody for the ECL Detection System (Amersham). As a result, the *DDEFL1* protein was detected on western blots with an anti-myc antibody (Fig. 3a).

Furthermore, immunocytochemical staining was performed as follows. First, the cells were fixed with PBS containing 4% paraformaldehyde for 15 min, then rendered permeable with PBS containing 0.1% Triton X-100 for 2.5 min at RT. Subsequently the cells were covered with 2% BSA in PBS for 24 h at 4°C to block non-specific hybridization. Mouse anti-myc monoclonal antibody (Sigma) at 1:1000 dilution or mouse anti-FLAG antibody (Sigma) at 1:2000 dilution was used for the first antibody, and the reaction was visualized after incubation with Rhodamine-conjugated anti-mouse second antibody (Leinco and ICN). Nuclei were counter-stained with 4',6'-diamidine-2'-phenylindole dihydrochloride (DAPI). Fluorescent images were obtained under an ECLIPSE E800 microscope. The microscopic analysis indicated that the protein was present mainly in the cytoplasm (Fig. 3b). *DDEFL1* was also localized in the cytoplasm of human embryonal kidney (HEK293) cells (ATCC).

1-4. Effect of DDEFL1 on cell growth

The coding sequence of *DDEFL1* was cloned into the pcDNA 3.1 vector (Invitrogen). NIH3T3 cells (ATCC) plated onto 10-cm dishes ($2X10^5$ cells/dish) were transfected with the resulting plasmid expressing *DDEFL1* (pcDNA-*DDEFL1*) and the control plasmid (pcDNA-*LacZ*) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution (Sigma), and further with an appropriate concentration of geneticin for two weeks. The cells were then fixed with 100% methanol and stained by Giemsa solution. Cells transfected with pcDNA-*DDEFL1* produced markedly more colonies than control cells. An increase in colony formation similarly occurred with transfected human hepatoma SNU423 (Korea cell-line bank) and Alexander (ATCC) cells, in which endogenous expression of *DDEFL1* is very low (Fig. 4a).

To investigate this growth-promoting effect further, NIH3T3 cells that stably expressed exogeneous *DDEFL1* were established. pDNA-myc/His-*DDEFL1* was transfected into NIH3T3 cells using FuGENE6 reagent (Boehringer) according to the supplier's recommendations. Twenty-four hours after transfection, geneticin was added to the cultures and single colonies were selected two weeks after transfection. Expression of *DDEFL1* was determined by semi-quantitative RT-PCR (Fig.4b). The growth rate of NIH3T3-*DDEFL1* cells was statistically higher than that of mock (NIH3T3-*LacZ*) cells in culture media containing 10% FBS ($P<0.05$) (Fig. 4c). In media containing only 0.1% FBS, NIH3T3-*DDEFL1* cells survived for 6 days, while control NIH3T3 cells died within 6 days under the same conditions. In this case, growth of NIH3T3-*DDEFL1* cells was statistically higher than that of mock cells in culture media containing 0.1% FBS ($P<0.01$) (Fig. 4d).

1-5. Suppression of *DDEFL1* expression in human hepatoma SNU475 cells by antisense S-oligonucleotides

The following six pairs of control (sense) and antisense S-oligonucleotides corresponding to the *DDEFL1* gene were

synthesized.

Antisense:

DDEFL1-AS1 5'-TGCTCCGGCATGGCGG-3' (SEQ ID NO: 13);
DDEFL1-AS2 5'-GCTGAACTGCTCCGGC-3' (SEQ ID NO: 14);
DDEFL1-AS3 5'-TCCAAGATCTCCTCCC-3' (SEQ ID NO: 15);
DDEFL1-AS4 5'-TCTCCTTCCAAGATCT-3' (SEQ ID NO: 16);
DDEFL1-AS5 5'-GCGCTGAGCCGGCCTC-3' (SEQ ID NO: 17); and
DDEFL1-AS6 5'-CCTCACCTCCTCCCGC-3' (SEQ ID NO: 18).

Control:

DDEFL1-S1 5'-CCGCCATGCCGGAGCA-3' (SEQ ID NO: 19);
DDEFL1-S2 5'-GCCGGAGCAGTTCAGC-3' (SEQ ID NO: 20);
DDEFL1-S3 5'-GGGAGGAGATCTTGGA-3' (SEQ ID NO: 21);
DDEFL1-S4 5'-AGATCTTGGAAGGAGA-3' (SEQ ID NO: 22);
DDEFL1-S5 5'-GAGGCCGGCTCAGCGC-3' (SEQ ID NO: 23); and
DDEFL1-S6 5'-GCGGGAGGAGGTGAGG-3' (SEQ ID NO: 24).

Using LIPOFECTIN Reagent (GIBCO BRL), the synthetic S-oligonucleotides were transfected into SNU475 cells (Korea cell-line bank), which had shown the highest level of *DDEFL1* expression among the six hepatoma cell lines we examined (data not shown). Twelve and twenty-four hours after transfection, antisense S-oligonucleotides AS1 and AS5 significantly suppressed expression of *DDEFL1* compared to the respective control S-oligonucleotides S1 and S5 (Fig. 5a). Six days after transfection, surviving cells transfected with antisense S-oligonucleotide AS1 or AS5 were markedly fewer than cells transfected with the respective control S-oligonucleotide S1 or S5 (Fig. 5b). Consistent results were obtained in three independent experiments.

Example 2

2-1. Identification of *VANGL1* commonly up-regulated in human hepatocellular carcinomas

The genome-wide cDNA microarray analysis carried out in Example 1 also revealed that a gene with an in-house accession

number of *D3244* corresponding to an EST (Hs.122730) of a UniGene cluster, was found to be significantly up-regulated in ten of twelve clinical HCCs compared with the corresponding non-cancerous liver tissues. The relative expression ratio compared to corresponding non-cancerous tissue of these 12 tumors ranged from 1.5 to 16.0 (Fig. 6a). Up-regulated expression (Cy3:Cy5 intensity ratio, >2.0) was observed in 10 of the 12 HCCs that passed through the cutoff filter (both Cy3 and Cy5 signals greater than 25,000). The elevated expression of *D3244* was also confirmed in ten additional HCC cases by semi-quantitative RT-PCR performed similarly to Example 1-1 using the primer set, forward: 5'- GAGTTGTATTATGAAGAGGCCGA (SEQ ID NO: 25); reverse: 5'- ATGTCTCAGACTGTAAGCGAAGG (SEQ ID NO: 26) (Fig. 6b).

2-2. Expression of *VANGL1* in human adult tissues

Multi-tissue northern blot analysis using *D3244* cDNA as a probe was performed in the same manner as in Example 1-2 and the results showed a 1.9-kb transcript abundantly expressed in testis and ovary in a tissue-specific manner (Fig. 7a). NCBI database search for genomic sequences corresponding to *D3244* found two sequences (GenBank accession number: AL450389 and AL592436) assigned to chromosomal band 1p22. Using GENSCAN, and Gene Recognition and Assembly Internet Link program, candidate-exon sequences were predicted and exon-connection was performed. In addition, 5' RACE was carried out using a reverse primer (5'-TGTCAGCTCTCCGCTTGCGGAAAAAAAG (SEQ ID NO: 27)) to determine the sequence of the 5' region of the transcript in the same manner as in Example 1-2. As a result, an assembled human cDNA sequence of 1879 nucleotides containing an open reading frame of 1572 nucleotides (GenBank accession number: AB057596) was obtained. The predicted amino acid sequence shared 40% and 63% identity with *strabismus* (*Van Gogh*) and *VANGL2*. Hence, the gene corresponding *D3244* was termed as *Van Gogh Like 1* (*VANGL1*). Simple Modular Architecture Research Tool suggested that the predicted protein contained putative four transmembrane domains (codons 111-133, 148-170, 182-204,

219-241) (Fig. 7b).

2-3. Subcellular localization of VANGL1

The pcDNA3.1-myc/His-*VANGL1* plasmid expressing c-myc-tagged *VANGL1* protein was transiently transfected into SNU475 cells (Korea cell-line bank). Immunocytochemical staining was performed in the same manner as in Example 1-3. The results revealed that the tagged *VANGL1* protein was present in the cytoplasm (Fig. 8a and 8b).

2-4. Growth suppression of hepatoma cells by antisense S-oligonucleotides designated to reduce expression of *VANGL1*

To test whether suppression of *VANGL1* may result in cell cycle arrest and/or cell death of HCC cells, the following four pairs of antisense and control (sense) S-oligonucleotides were synthesized and transfected into SNU475 cells.

Antisense:

antisense 1 5'-GTATCCATAGCAATGG-3' (SEQ ID NO: 28);
antisense 2 5'-TGGATTGGGTATCCAT-3' (SEQ ID NO: 29);
antisense 3 5'-TAAGTGGATTGGGTAT-3' (SEQ ID NO: 30); and
antisense 4 5'-ACTCCTACCTGCCTGT-3' (SEQ ID NO: 31).

Control:

sense 1 5'-CCATTGCTATGGATAC-3' (SEQ ID NO: 32);
sense 2 5'-ATGGATACCCAATCCA-3' (SEQ ID NO: 33);
sense 3 5'-ATACCCAATCCACTTA-3' (SEQ ID NO: 34); and
sense 4 5'-ACAGGCAGGTAGGAGT-3' (SEQ ID NO: 35).

Antisense S-oligonucleotide encompassing the initiation codon (antisense 3) significantly decreased endogenous expression of *VANGL1* in SNU475 cells (Fig. 9a).

Cell viability was evaluated by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as follows. Cells were plated at a density of $5X10^5$ cells/100 mm dish. At 24 hours after seeding, the cells were transfected in triplicate with sense or antisense S-oligonucleotide

designated to suppress *VANGL1*. At 72 hours after transfection, the medium was replaced with fresh medium containing 500 μg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) and the plates were incubated for four hours at 37°C. Subsequently, the cells were lysed by the addition of 1 ml of 0.01 N HCl/10%SDS and absorbance of lysates was measured with an ELISA plate reader at a test wavelength of 570 nm (reference, 630 nm). The cell viability was represented by the absorbance compared to that of control cells.

Transfection of the antisense S-oligonucleotide, antisense 3, significantly reduced number of surviving cells compared with control sense S-oligonucleotide, sense 3 (Fig. 9b and 9c). This result was confirmed by three independent experiments.

Furthermore, flow cytometry analysis was performed as follows. Cells were plated at a density of $1X10^5$ cells/100 mm dish and trypsinized at the given time course, followed by fixation in 70% cold ethanol. After RNase treatment, cells were stained with propidium iodide (50 μg/ml) in PBS. Flow cytometry was performed on a Becton Dickinson FACScan and analyzed by CellQuest and ModFit software (Verity Software House). The percentages of nuclei in G0/G1, S and G2/M phases of the cell cycle, and any sub-G1 population were determined from at least 20,000 ungated cells.

FACS analysis demonstrated that inhibition of *VANGL1* significantly increased number of cells at sub-G1 phase (Fig. 9d). These results suggest that *VANGL1* may play an important role for cell growth and/or survival of hepatocellular carcinoma cells.

Example 3

3-1. *LGN* is commonly increased in human hepatocellular carcinomas

Among commonly up-regulated genes by the microarray analysis performed in Example 1-1, a gene, *D3636* corresponding to *LGN* (GenBank accession number: U54999) was selected because it was significantly up-regulated in ten of twelve clinical

HCCs compared with the corresponding non-cancerous liver tissues. The relative expression ratio compared to corresponding non-cancerous tissue of these 12 tumors ranged from 0.7 to 16.0. Up-regulated expression of *LGN* (Cy3:Cy5 intensity ratio, >2.0) was observed in 10 of the 12 HCCs that passed through the cutoff filter (both Cy3 and Cy5 signals greater than 25,000) (Fig. 10a). The elevated expression of *LGN* was also confirmed in additional ten HCC cases by semi-quantitative RT-PCR performed using a primer set, forward: 5'-ATCTGAAGCACTTAGCAATTGC (SEQ ID NO: 36), reverse: 5'-CTGTAGCTCAGACCAAGAACC (SEQ ID NO: 37), similarly to Example 1-1 (Fig. 10b).

3-2. Genomic structure of *LGN*

LGN cDNA consists of 2,336 nucleotides and encodes a 677 amino acid peptide. Comparison of the cDNA sequence with genomic sequences disclosed that the *LGN* gene consists of 14 exons (Fig. 11).

3-3. Subcellular localization of *LGN*

The pcDNA3.1-myc/His-*LGN* plasmid expressing c-myc-tagged *LGN* protein was transiently transfected into COS7 cells. A 72 kDa-band corresponding to myc-tagged *LGN* protein was detected by immunoblot analysis in the same manner as in Example 1-3 (Fig. 12). Similarly, immunocytochemical staining was performed as in Example 1-3 and the results revealed that the tagged *LGN* protein was present in the cytoplasm and nucleus in the cells.

3-4. LGN gene transfer can promote cell growth

To analyze the effect of *LGN* on cell growth, a colony-formation assay was carried out as in Example 1-4 by transfecting NIH3T3, SNU423, Alexander and SNU475 cells with a plasmid expressing *LGN* (pcDNA3.1-myc/His-*LGN*). Compared with a control plasmid (pcDNA3.1-myc/His-*LacZ*), pcDNA3.1-myc/His-*LGN* produced markedly a larger number of colonies in these cells (Fig. 13a). This result was confirmed

by three independent experiments.

To further investigate the effect of *LGN* on cell growth, NIH3T3 cells that stably expressed exogeneous *LGN* (NIH3T3-*LGN* cells) were established. NIH3T3-*LGN* cells showed higher growth rate than control NIH3T3-*LacZ* cells (Fig. 13b).

3-5. Antisense S-oligonucleotides of *LGN* suppressed growth of human hepatoma SNU475 cells

The following five pairs of control (sense) and antisense S-oligonucleotides corresponding to *LGN* were synthesized and then transfected into SNU423 cells.

Antisense:

antisense 1 5'-CCATCGAGTCATATTA -3'(SEQ ID NO: 38);
antisense 2 5'-TTCCTCCATCGAGTCA -3'(SEQ ID NO: 39);
antisense 3 5'-AAATTTTCCTCCATCG -3'(SEQ ID NO: 40);
antisense 4 5'-AGTCTTACCTGTAACG -3'(SEQ ID NO: 41); and
antisense 5 5'-GCTTCCATTCTACAAA -3'(SEQ ID NO: 42).

Sense:

sense 1 5'-TAATATGACTCGATGG-3'(SEQ ID NO: 43);
sense 2 5'-TGACTCGATGGAGGAA-3'(SEQ ID NO: 44);
sense 3 5'-CGATGGAGGAAAATTT-3'(SEQ ID NO: 45);
sense 4 5'-CGTTACAGGTAAGACT-3'(SEQ ID NO: 46); and
sense 5 5'-TTTGTAGAATGGAAGC-3'(SEQ ID NO: 47).

The antisense S-oligonucleotide encompassing the initiation codon (antisense 3) significantly suppressed expression of *LGN* compared to control S-oligonucleotide (sense 3) 12 hours after transfection (Fig. 14a). Six days after transfection, the number of surviving cells transfected with antisense 3 were markedly fewer than that with control sense 3 (Fig. 14b). Consistent results were obtained in three independent experiments.

Industrial Applicability

The present invention provides cDNA nucleotide sequences and polypeptide amino acid sequence of *DDEFL1*, *VANGL1* or *LGN*, which have been found to be commonly up-regulated in hepatocellular carcinomas. Thus, these polypeptides can be used as markers to determine the presence or absence of liver cancers. The information of these nucleotide sequences enables one to design probes and primers to detect or amplify the *DDEFL1*, *VANGL1* or *LGN* genes. It also enables synthesis of antisense nucleotide sequence that inhibits expression of the *DDEFL1*, *VANGL1* or *LGN* polypeptides. The amino acid sequence information enables one to prepare antibodies that bind to the *DDEFL1*, *VANGL1* or *LGN* polypeptides. The probes and primers as well as the antibodies are useful as a reagent for detecting hepatocellular carcinomas. Furthermore, the present inventors demonstrated that suppressing the expression of *DDEFL1*, *VANGL1* or *LGN* by antisense oligonucleotides markedly decreases growth of HCC cells. Thus, the antisense oligonucleotides can be used to inhibit growth of HCC cells. The present invention also contributes to further clarify the mechanisms of hepatocellular carcinogenesis and to discover molecular targets for development of effective drugs to treat liver cancers.

CLAIMS

1. An isolated nucleic acid selected from the group consisting of:

(a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 1 or NO: 3;

(b) a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or NO: 4;

(c) a nucleic acid comprising a strand that hybridizes under high stringent conditions to a nucleotide sequence consisting of SEQ ID NO: 1 or NO: 3 or the complement thereof.

2. An isolated polypeptide selected from the group consisting of:

(d) a polypeptide encoded by the nucleotide sequence of SEQ ID NO: 1 or NO: 3;

(e) a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or NO: 4;

(f) a polypeptide having at least 65% identity to SEQ ID NO: 2 or NO: 4.

3. A vector carrying the nucleic acid of claim 1.

4. A transformant carrying the nucleic acid of claim 1 or the vector of claim 3.

5. A method of producing a polypeptide, the method comprising culturing the transformant of claim 4 in a culture, expressing the polypeptide in the transformant, and recoverying the polypeptide from the culture.

6. An antibody that specifically binds to the polypeptide of claim 2.

7. A method for detecting hepatoceullar carcinoma, the method comprising the steps of:

(a) preparing a biological sample from a subject;

(b) measuring the expression level of at least one of polypeptides selected from the group consisting of the polypeptide of SEQ ID NO: 1, a polypeptide of SEQ ID NO: 3, and the polypeptide of SEQ ID NO: 5;

(c) comparing the expression level with that measured

in a non-cancerous sample; and

(d) determining the presence or absence of the cancer in the subject.

8. A reagent for detecting hepatocellular carcinomas, comprising a nucleic acid comprising a strand that hybridizes under high stringent conditions to a nucleotide sequence consisting of SEQ ID NO: 1, NO: 3, or NO: 5 or the complement thereof.

9. A reagent for detecting hepatocellular carcinomas, comprising the antibody of claim 6.

10. A method for inhibiting growth of hepatocellular carcinomas, the method comprising introducing at least one of antisense oligonucleotides that hybridizes with the nucleotide sequence of SEQ ID NO: 1, NO: 3, or NO: 5 into hepatocelluar carcinomas.

Fig. 1

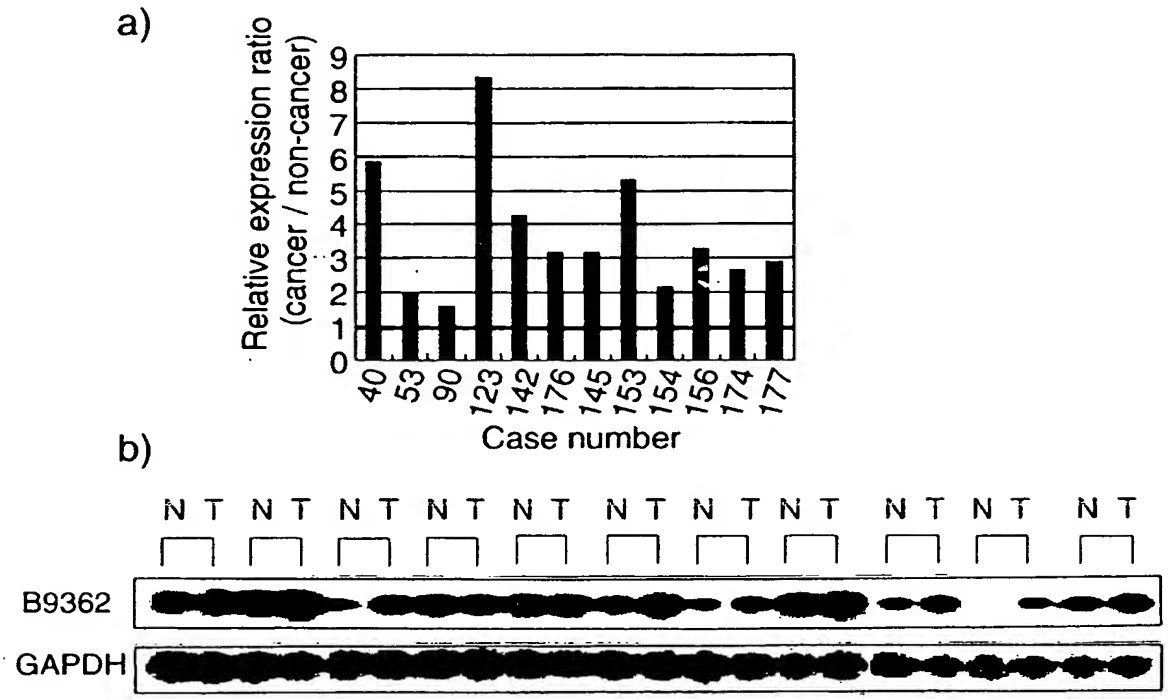

a)
Relative expression ratio
(cancer / non-cancer)
0
1
2
3
4
5
6
7
8
9
40
53
90
123
142
176
145
153
154
156
174
177
Case number
b)
N T N T N T N T N T N T N T N T N T N T N T
B9362
GAPDH

Fig. 2

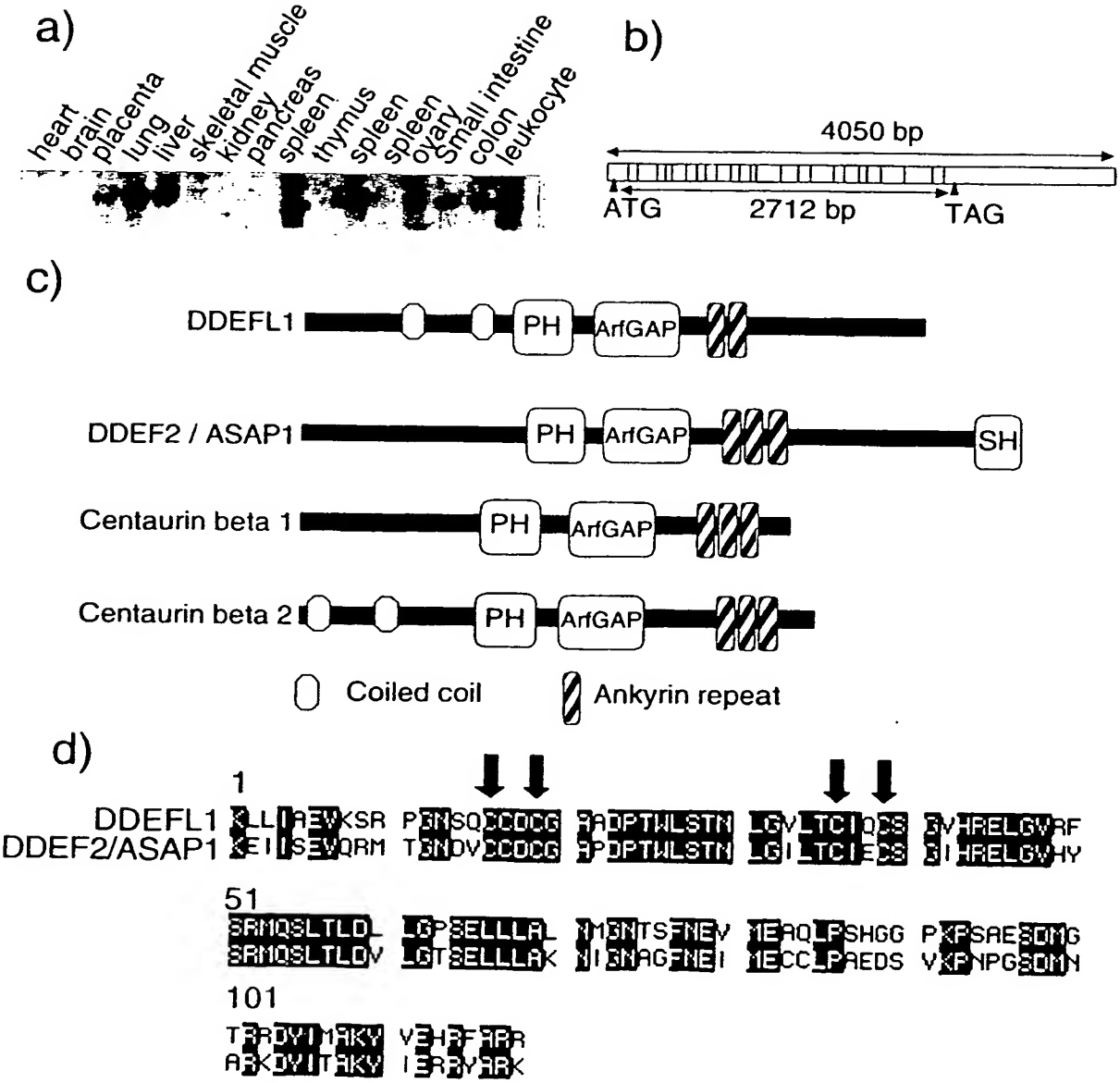

a)
heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
spleen
thymus
spleen
spleen
ovary
Small intestine
colon
leukocyte
b)
4050 bp
ATG
2712 bp
TAG
c)
DDEFL1
PH
ArfGAP
DDEF2 / ASAP1
SH
Centaurin beta 1
Centaurin beta 2
Coiled coil
Ankyrin repeat
d)
1
DDEFL1
DDEF2/ASAP1
51
101

Fig. 3

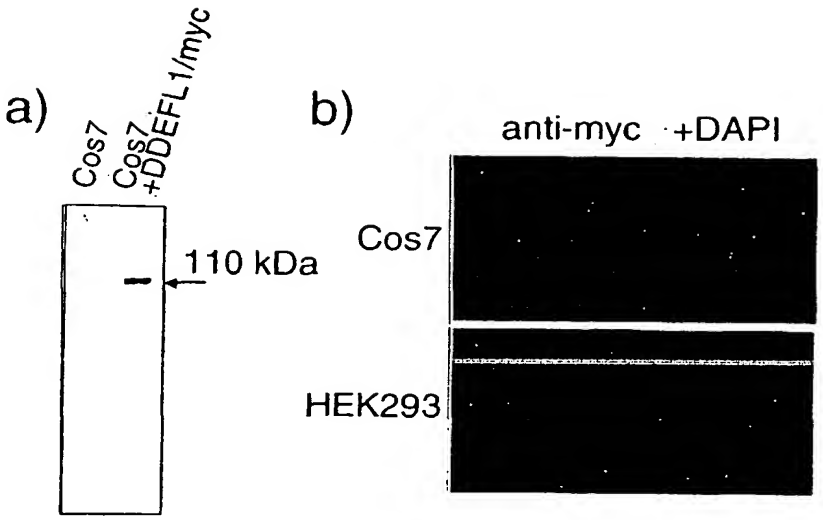


Fig. 4

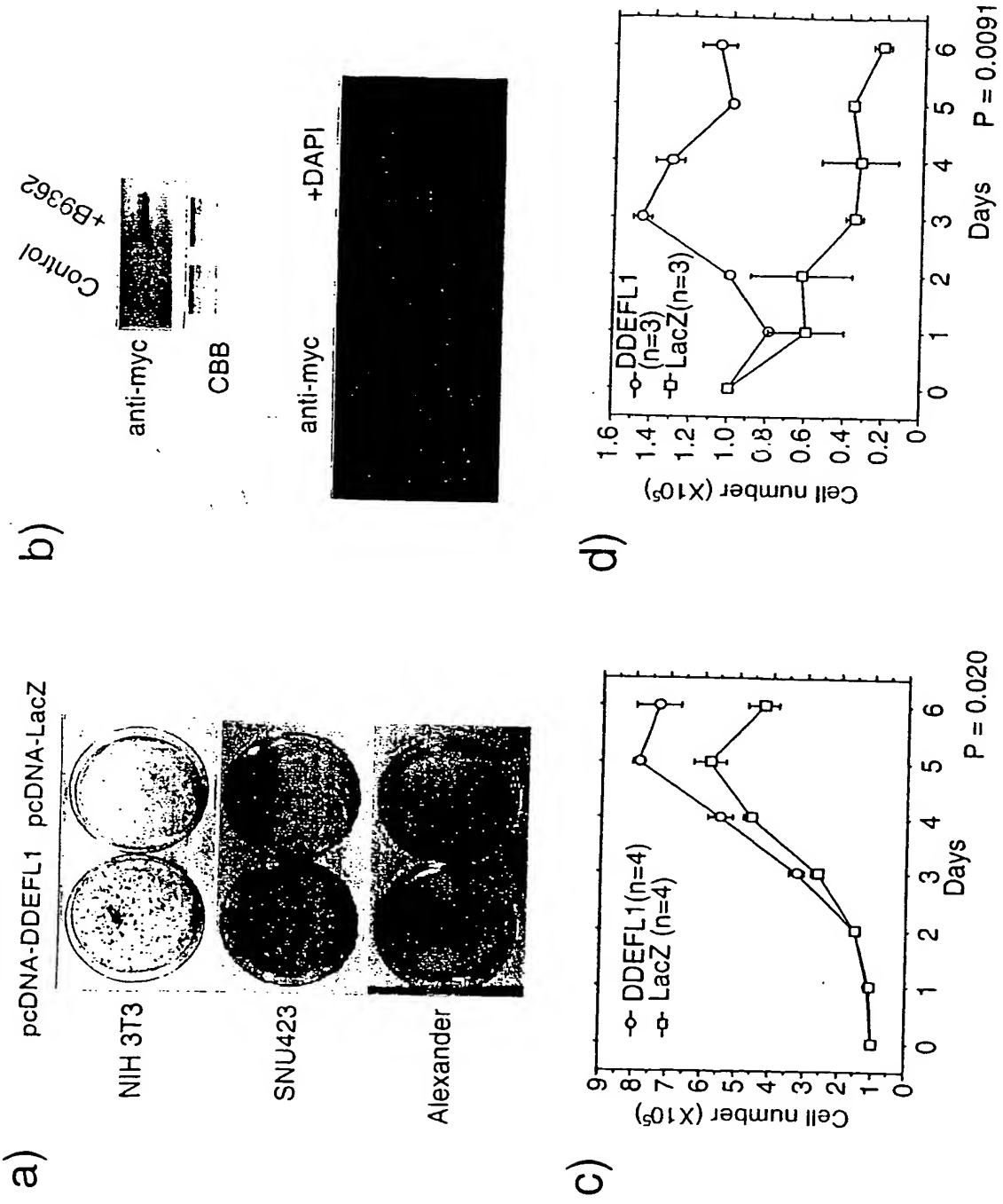

a)
pcDNA-DDEFL1
pcDNA-LacZ
NIH 3T3
SNU423
Alexander
b)
Control
+B9362
anti-myc
CBB
anti-myc
+DAPI
c)
DDEFL1(n=4)
LacZ (n=4)
Cell number (X10⁵)
Days
P = 0.020
d)
DDEFL1 (n=3)
LacZ(n=3)
Cell number (X10⁵)
Days
P = 0.0091

Fig. 5

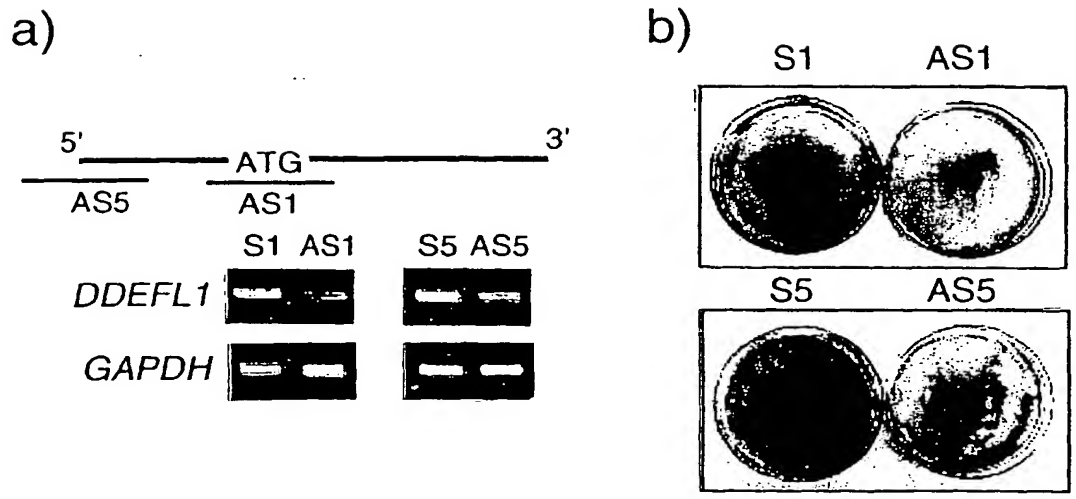
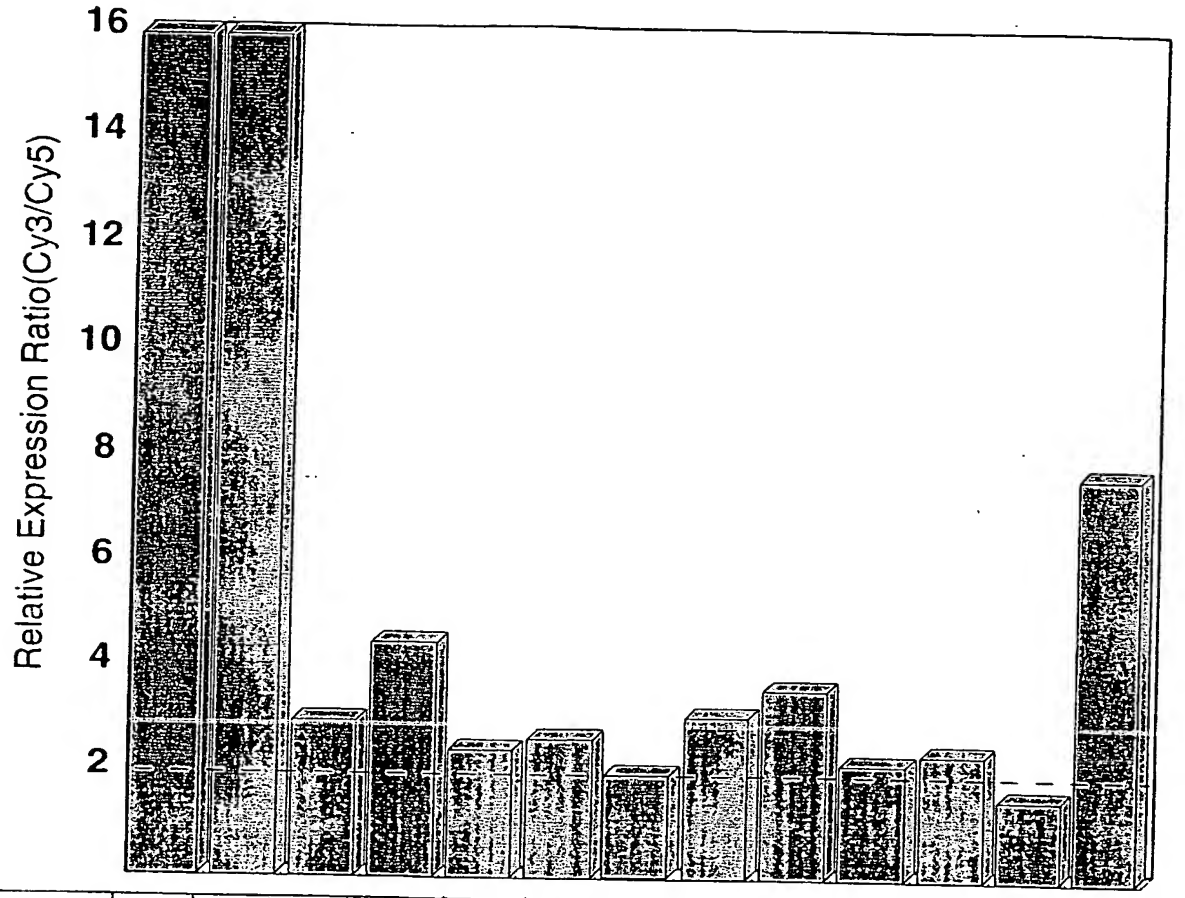


Fig. 6

a



Case No.	53	90	104	123	143	145	146	148	153	154	161	174	177
Ratio	16.00	16.00	2.991	4.481	2.460	2.656	1.970	3.098	3.631	2.206	2.387	1.551	7.733

b

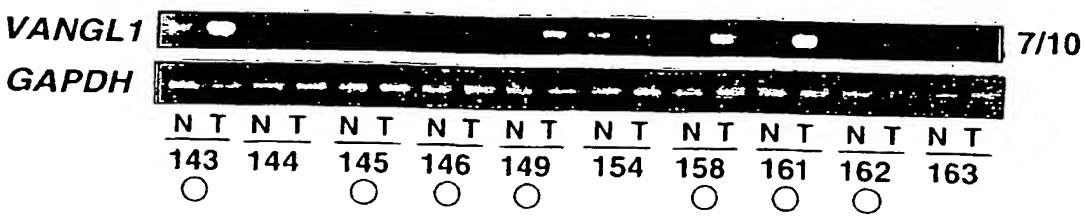


Fig. 7

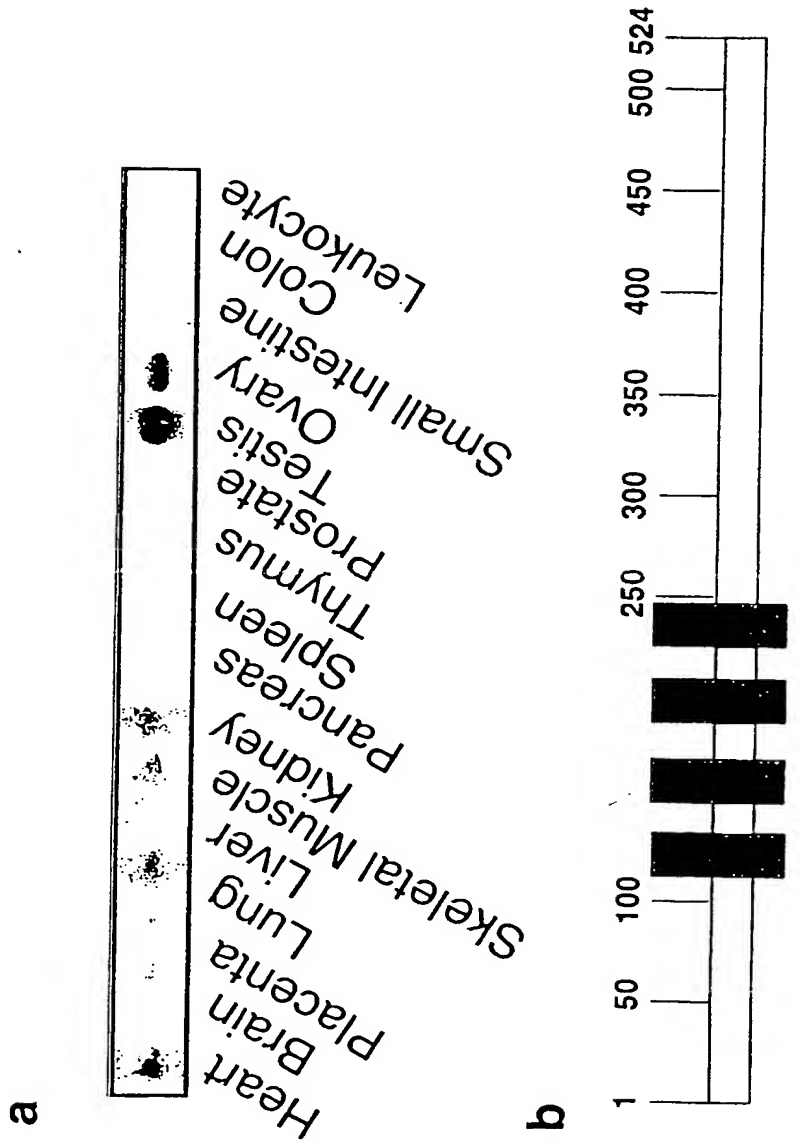
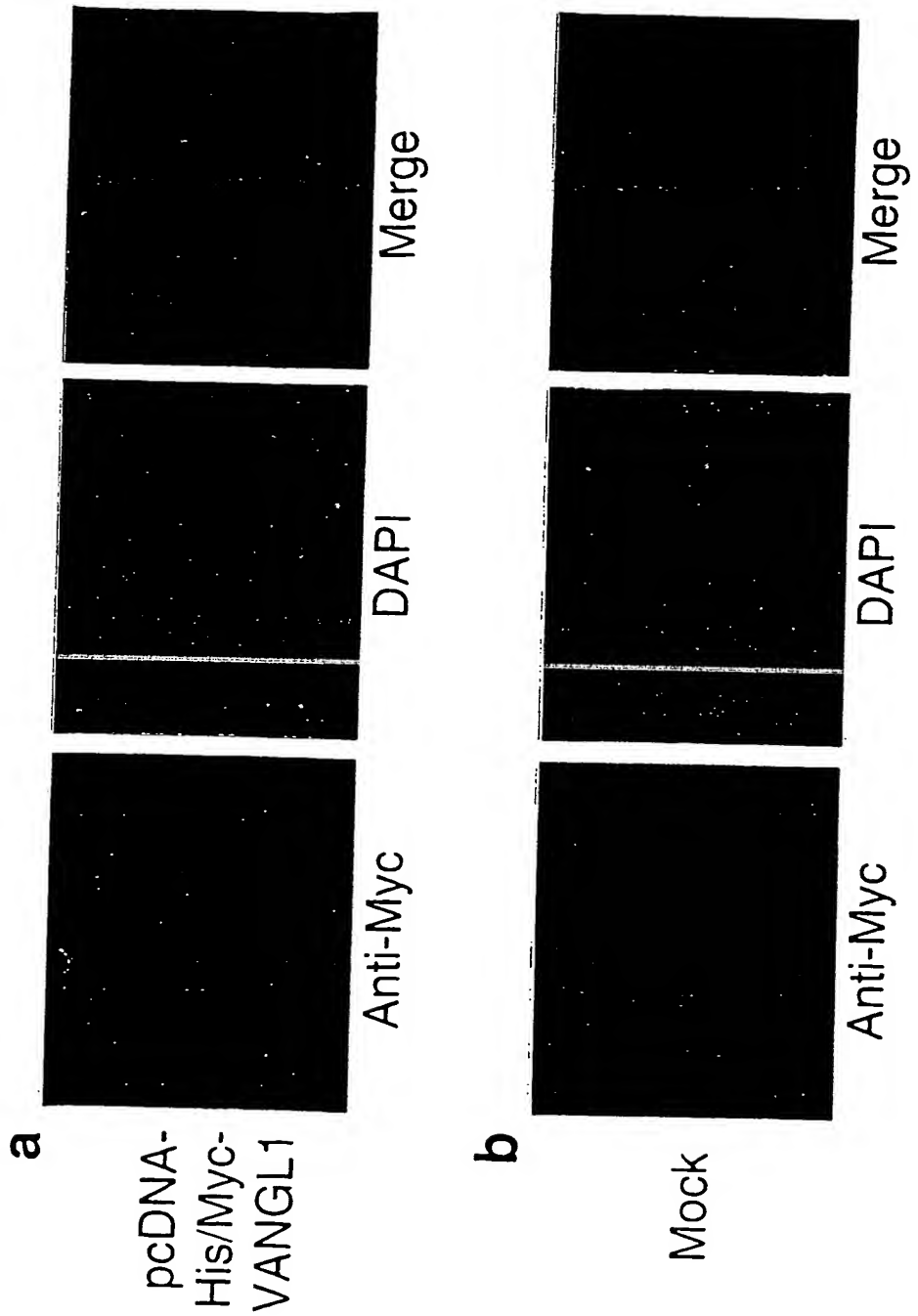

a
Heart
Brain
Placenta
Lung
Liver
Skeletal Muscle
Kidney
Pancreas
Spleen
Thymus
Prostate
Testis
Ovary
Small Intestine
Colon
Leukocyte
b
524
500
450
400
350
300
250
100
50
1

Fig. 8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pcDNA-His/Myc-VANGL1
Anti-Myc
DAPI
Merge
b
Mock
Anti-Myc
DAPI
Merge

Fig. 9

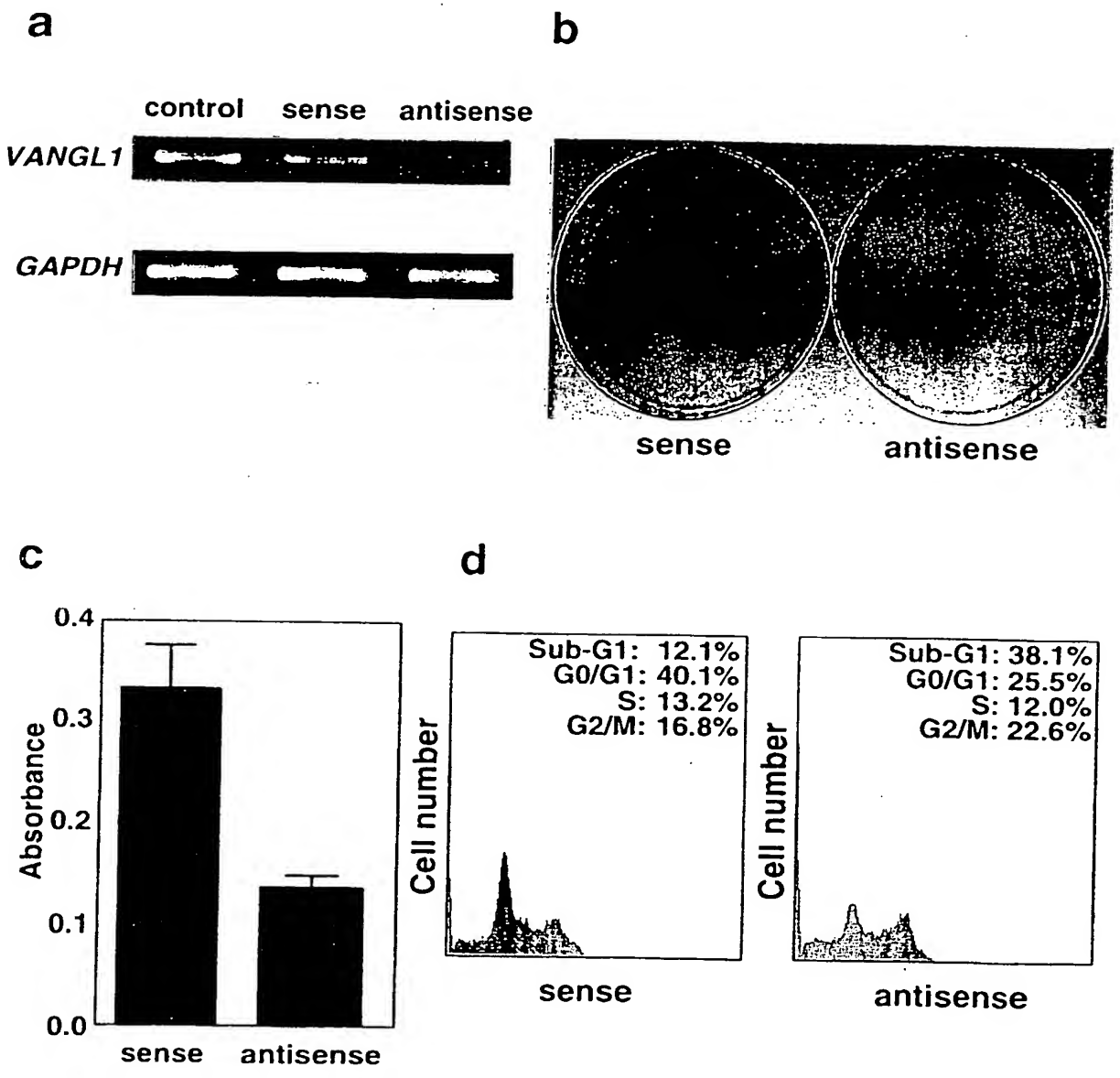
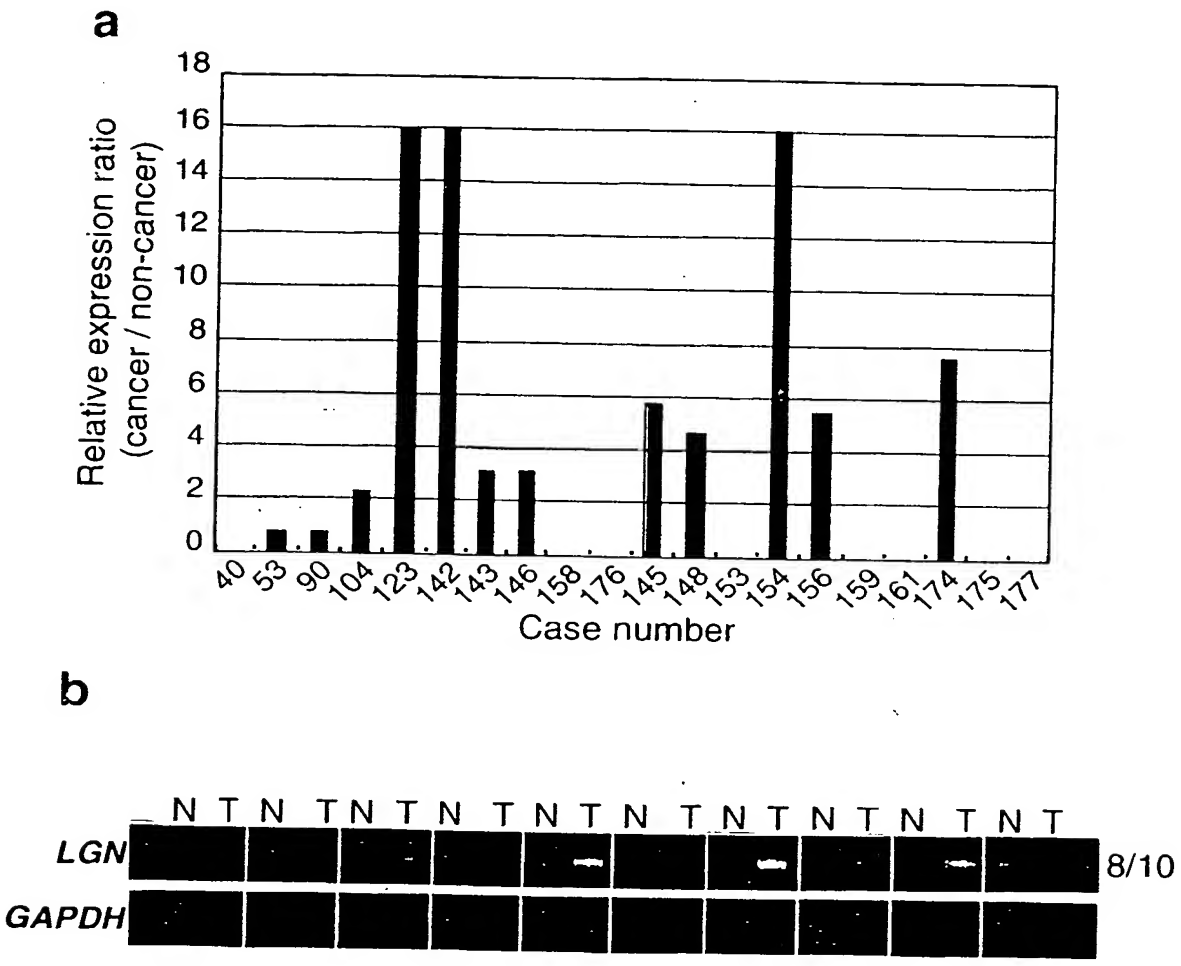

a
control
sense
antisense
VANGL1
GAPDH
b
sense
antisense
c
Absorbance
0.4
0.3
0.2
0.1
0.0
sense
antisense
d
Cell number
Sub-G1: 12.1%
G0/G1: 40.1%
S: 13.2%
G2/M: 16.8%
sense
Cell number
Sub-G1: 38.1%
G0/G1: 25.5%
S: 12.0%
G2/M: 22.6%
antisense

Fig. 10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Relative expression ratio (cancer / non-cancer)
0
2
4
6
8
10
12
14
16
18
40 53 90 104 123 142 143 146 158 176 145 148 153 154 156 159 161 174 175 177
Case number
b
N T N T N T N T N T N T N T N T N T N T
LGN
GAPDH
8/10

Fig. 11

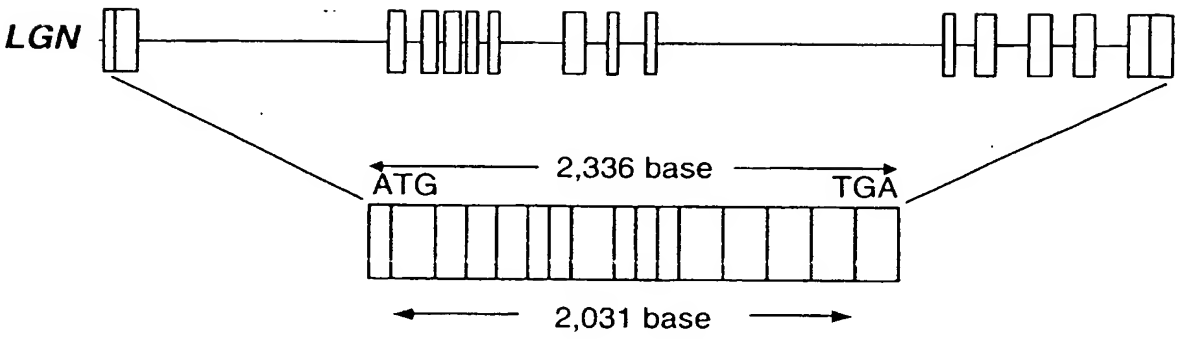


Fig. 12

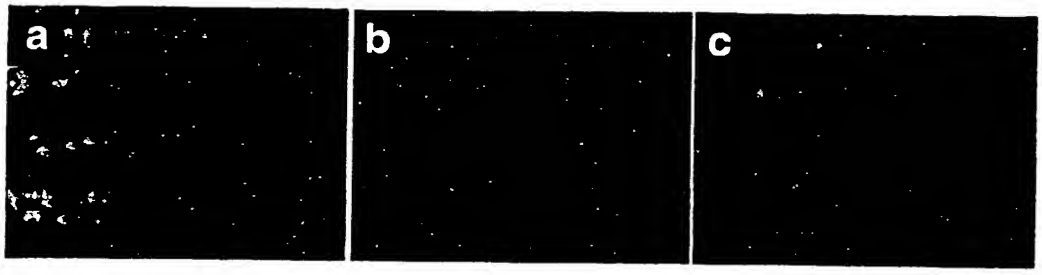
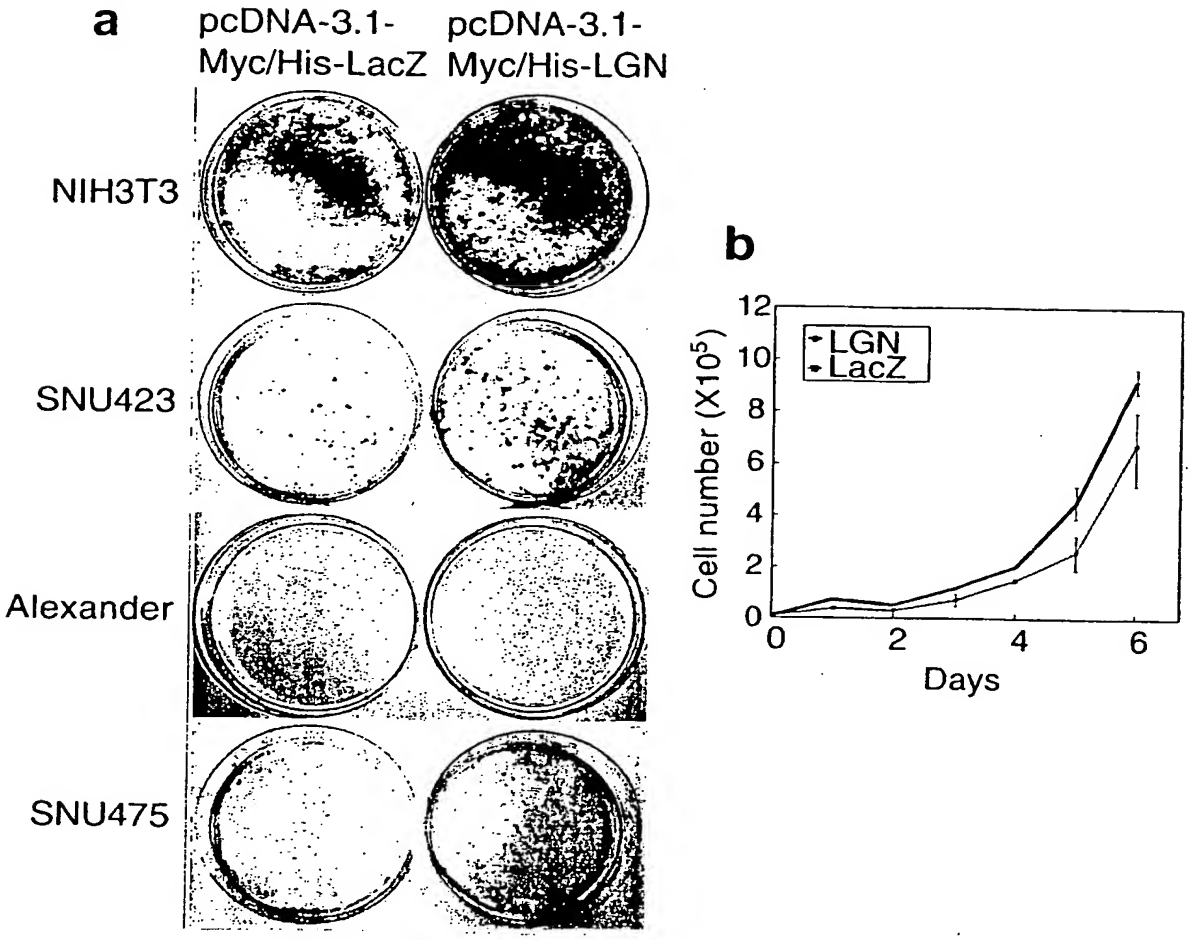


Fig. 13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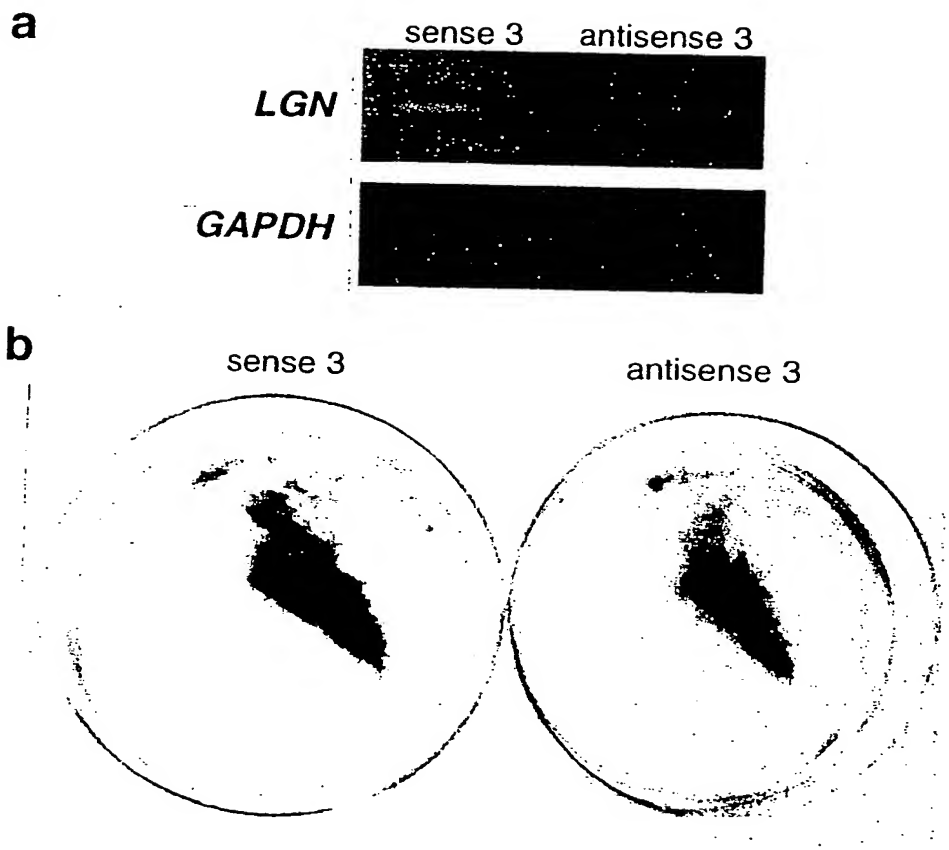
pcDNA-3.1-Myc/His-LGN
NIH3T3
SNU423
Alexander
SNU475
b
LGN
LacZ
Cell number (X10^5)
Days
0
2
4
6
8
10
12

Fig. 14



SEQUENCE LISTING

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ONCOTHERAPY SCIENCE, INC.

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gtcttaaggg ttgggcttgg gaataggcag ggtcagattc cagggcatgg ctctggactc 3360

agctggttta tacctatatg accattacag ttgtctacag atcacatcca ttctggctgg 3420

tcaacatgca tgctgtactg gctgttaaat aaaaatattc tgaatgtcac tccttttgag 3480

ggacagcaca gccttcccta ggcattctcc tatattcccc agccaaattg tagagtcaga 3540

tgcacccaca tttgcctgtg tccttgattt agcaggaagg aaaggaatag tcggggttga 3600

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atggagaggc aaagaaagaa gggagagtaa tagaattggg agggcagaga cttaagggtt 3720

ctgcttccca gccctagaaa ttctatcatt gctcagcccc aatgagaaag cagatacacc 3780

taagccatca tcaaccacta acatctcaac ttgccagttg ctgggtgctg ggccctggca 3840

ggaatgggcc aagccaagca ggggagacta gagagcacca atggccaaca cagctgcctg 3900

gctggggagg ctgtgctgtt tcccctggag acctgactgg tctgtggttc ccacaggaac 3960

agggttgtct tttgagcccc cagtgtctgg tttcattcat ctcagacttg ttatttcact 4020

catctctaat aaaggattgg ggggtcagtt 4050

<210> 2
<211> 903
<212> PRT
<213> Homo sapiens

<400> 2
Met Pro Glu Gln Phe Ser Val Ala Glu Phe Leu Ala Val Thr Ala Glu
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Asp Leu Ser Ser Pro Ala Gly Ala Ala Ala Phe Ala Ala Lys Met Pro
20 25 30

Arg Tyr Arg Gly Ala Ala Leu Ala Arg Glu Glu Ile Leu Glu Gly Asp
35 40 45

Gln Ala Ile Leu Gln Arg Ile Lys Lys Ala Val Arg Ala Ile His Ser
50 55 60

Ser Gly Leu Gly His Val Glu Asn Glu Glu Gln Tyr Arg Glu Ala Val
65 70 75 80

Glu Ser Leu Gly Asn Ser His Leu Ser Gln Asn Ser His Glu Leu Ser
85 90 95

Thr Gly Phe Leu Asn Leu Ala Val Phe Thr Arg Glu Val Ala Ala Leu
100 105 110

Phe Lys Asn Leu Ile Gln Asn Leu Asn Asn Ile Val Ser Phe Pro Leu
115 120 125

Asp Ser Leu Met Lys Gly Gln Leu Arg Asp Gly Arg Gln Asp Ser Lys
130 135 140

Lys Gln Leu Glu Lys Ala Trp Lys Asp Tyr Glu Ala Lys Met Ala Lys
145 150 155 160

Leu Glu Lys Glu Arg Asp Arg Ala Arg Val Thr Gly Gly Ile Pro Gly
165 170 175

Glu Val Ala Gln Asp Met Gln Arg Glu Arg Arg Ile Phe Gln Leu His
180 185 190

Met Cys Glu Tyr Leu Leu Lys Ala Gly Glu Ser Gln Met Lys Gln Gly
195 200 205

Pro Asp Phe Leu Gln Ser Leu Ile Lys Phe Phe His Ala Gln His Asn
210 215 220

Phe Phe Gln Asp Gly Trp Lys Ala Ala Gln Ser Leu Phe Pro Phe Ile
225 230 235 240

Glu Lys Leu Ala Ala Ser Val His Ala Leu His Gln Ala Gln Glu Asp
245 250 255

Glu Leu Gln Lys Leu Thr Gln Leu Arg Asp Ser Leu Arg Gly Thr Leu
260 265 270

Gln Leu Glu Ser Arg Glu Glu His Leu Ser Arg Lys Asn Ser Gly Cys
275 280 285

Gly Tyr Ser Ile His Gln His Gln Gly Asn Lys Gln Phe Gly Thr Glu
290 295 300

Lys Val Gly Phe Leu Tyr Lys Lys Ser Asp Gly Ile Arg Arg Val Trp
305 310 315 320

Gln Lys Arg Lys Cys Gly Val Lys Tyr Gly Cys Leu Thr Ile Ser His
325 330 335

Ser Thr Ile Asn Arg Pro Pro Val Lys Leu Thr Leu Leu Thr Cys Gln
340 345 350

Val Arg Pro Asn Pro Glu Glu Lys Lys Cys Phe Asp Leu Val Thr His
355 360 365

Asn Arg Thr Tyr His Phe Gln Ala Glu Asp Glu His Glu Cys Glu Ala
370 375 380

Trp Val Ser Val Leu Gln Asn Ser Lys Asp Glu Ala Leu Ser Ser Ala
385 390 395 400

Phe Leu Gly Glu Pro Ser Ala Gly Pro Gly Ser Trp Gly Ser Ala Gly
405 410 415

His Asp Gly Glu Pro His Asp Leu Thr Lys Leu Leu Ile Ala Glu Val
420 425 430

Lys Ser Arg Pro Gly Asn Ser Gln Cys Cys Asp Cys Gly Ala Ala Asp
435 440 445

Pro Thr Trp Leu Ser Thr Asn Leu Gly Val Leu Thr Cys Ile Gln Cys
450 455 460

Ser Gly Val His Arg Glu Leu Gly Val Arg Phe Ser Arg Met Gln Ser
465 470 475 480

Leu Thr Leu Asp Leu Leu Gly Pro Ser Glu Leu Leu Leu Ala Leu Asn
485 490 495

Met Gly Asn Thr Ser Phe Asn Glu Val Met Glu Ala Gln Leu Pro Ser
500 505 510

His Gly Gly Pro Lys Pro Ser Ala Glu Ser Asp Met Gly Thr Arg Arg
515 520 525

Asp Tyr Ile Met Ala Lys Tyr Val Glu His Arg Phe Ala Arg Arg Cys
530 535 540

Thr Pro Glu Pro Gln Arg Leu Trp Thr Ala Ile Cys Asn Arg Asp Leu
545 550 555 560

Leu Ser Val Leu Glu Ala Phe Ala Asn Gly Gln Asp Phe Gly Gln Pro
565 570 575

Leu Pro Gly Pro Asp Ala Gln Ala Pro Glu Glu Leu Val Leu His Leu
580 585 590

Ala Val Lys Val Ala Asn Gln Ala Ser Leu Pro Leu Val Asp Phe Ile
595 600 605

Ile Gln Asn Gly Gly His Leu Asp Ala Lys Ala Ala Asp Gly Asn Thr
610 615 620

Ala Leu His Tyr Ala Ala Leu Tyr Asn Gln Pro Asp Cys Leu Lys Leu
625 630 635 640

Leu Leu Lys Gly Arg Ala Leu Val Gly Thr Val Asn Glu Ala Gly Glu
645 650 655

Thr Ala Leu Asp Ile Ala Arg Lys Lys His His Lys Glu Cys Glu Glu
660 665 670

Leu Leu Glu Gln Ala Gln Ala Gly Thr Phe Ala Phe Pro Leu His Val
675 680 685

Asp Tyr Ser Trp Val Ile Ser Thr Glu Pro Gly Ser Asp Ser Glu Glu
690 695 700

Asp Glu Glu Glu Lys Arg Cys Leu Leu Lys Leu Pro Ala Gln Ala His
705 710 715 720

Trp Ala Ser Gly Arg Leu Asp Ile Ser Asn Lys Thr Tyr Glu Thr Val
725 730 735

Ala Ser Leu Gly Ala Ala Thr Pro Gln Gly Glu Ser Glu Asp Cys Pro
740 745 750

Pro Pro Leu Pro Val Lys Asn Ser Ser Arg Thr Leu Val Gln Gly Cys
755 760 765

Ala Arg His Ala Ser Gly Asp Arg Ser Glu Val Ser Ser Leu Ser Ser
770 775 780

Glu Ala Pro Glu Thr Pro Glu Ser Leu Gly Ser Pro Ala Ser Ser Ser
785 790 795 800

Ser Leu Met Ser Pro Leu Glu Pro Gly Asp Pro Ser Gln Ala Pro Pro
805 810 815

Asn Ser Glu Glu Gly Leu Arg Glu Pro Pro Gly Thr Ser Arg Pro Ser
820 825 830

Leu Thr Ser Gly Thr Thr Pro Ser Glu Met Tyr Leu Pro Val Arg Phe
835 840 845

Ser Ser Glu Ser Thr Arg Ser Tyr Arg Arg Gly Ala Arg Ser Pro Glu
850 855 860

Asp Gly Pro Ser Ala Arg Gln Pro Leu Pro Arg Arg Asn Val Pro Val
865 870 875 880

Gly Ile Thr Glu Gly Asp Gly Ser Arg Thr Gly Ser Leu Pro Ala Ser
885 890 895

Ser Val Gln Leu Leu Gln Asp
900

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<211> 1879
<212> DNA
<213> Homo sapiens

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ggagagcgca acaggcagaa tttgttcctg ttgaagagtg gctcctcttc taatttccag 120

actccttgag gttttaggag tctggtaggt gaaattttct acctctaagg agaaacagta 180

cctgctcctt cctcaagcgc aagccctcca ttgctatgga taccgaatcc acttattctg 240

gatattctta ctattcaagt cattcgaaaa aatctcacag acaaggggaa agaactagag 300

agagacacaa gtcaccccgg aataaagacg gcagagggtc agaaaagtct gtcaccattc 360

aacctcccac tggagagccc ctgttgggaa atgattctac tcggacagag gaagttcagg 420

atgacaactg gggagagacc accacggcca tcacaggcac ctcggagcac agcatatccc 480

aagaggacat tgccaggatc agcaaggaca tggaggacag cgtggggctg gattgcaaac 540

gctacctggg cctcaccgtc gcctcttttc ttggacttct agttttcctc acccctattg 600

ccttcatcct tttacctccg atcctgtgga gggatgagct ggagccttgt ggcacaattt 660

gtgaggggct ctttatctcc atggcattca aactcctcat tctgctcata gggacctggg 720

cacttttttt ccgcaagcgg agagctgaca tgccacgggt gtttgtgttt cgtgcccttt 780

tgttggtcct catctttctc tttgtggttt cctattggct tttttacggg gtccgcattt 840

tggactctcg ggaccggaat taccagggca ttgtgcaata tgcagtctcc cttgtggatg 900

ccctcctctt catccattac ctggccatcg tcctgctgga gctcaggcag ctgcagccca 960

tgttcacgct gcaggtggtc cgctccaccg atggcgagtc ccgcttctac agcctgggac 1020

acctgagtat ccagcgagca gcattggtgg tcctagaaaa ttactacaaa gatttcacca 1080

tctataaccc aaacctccta acagcctcca aattccgagc agccaagcat atggccgggc 1140

tgaaagtcta caatgtagat ggccccagta acaatgccac tggccagtcc cgggccatga 1200

ttgctgcagc tgctcggcgc agggactcaa gccacaacga gttgtattat gaagaggccg 1260

aacatgaacg gcgagtaaag aagcggaaag caaggctggt ggttgcagtg gaagaggcct 1320

tcatccacat tcagcgtctc caggctgagg agcagcagaa agccccaggg gaggtgatgg 1380

accctaggga ggccgcccag gccattttcc cctccatggc cagggctctc cagaagtacc 1440

tgcgcatcac ccggcagcag aactaccaca gcatggagag catcctgcag cacctggcct 1500

tctgcatcac caacggcatg acccccaagg ccttcctaga acggtacctc agtgcgggcc 1560

ccaccctgca atatgacaag gaccgctggc tctctacaca gtggaggctt gtcagtgatg 1620

aggctgtgac taatggatta cgggatggaa ttgtgttcgt ccttaagtgc ttggacttca 1680

gcctcgtagt caatgtgaag aaaattccat tcatcatact ctctgaagag ttcatagacc 1740

ccaaatctca caaatttgtc cttcgcttac agtctgagac atccgtttaa aagttctata 1800

tttgtggctt tattaaaaaa aaaagaaaaa tatatagaga gatatgcaaa aaaaataaaa 1860

gacaaaaaca aaaaaaaaa 1879

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<211> 524
<212> PRT
<213> Homo sapiens

<400> 4

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Ser Lys Lys Ser His Arg Gln Gly Glu Arg Thr Arg Glu Arg His Lys
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Ser Pro Arg Asn Lys Asp Gly Arg Gly Ser Glu Lys Ser Val Thr Ile
35 40 45

Gln Pro Pro Thr Gly Glu Pro Leu Leu Gly Asn Asp Ser Thr Arg Thr
50 55 60

Glu Glu Val Gln Asp Asp Asn Trp Gly Glu Thr Thr Thr Ala Ile Thr
65 70 75 80

Gly Thr Ser Glu His Ser Ile Ser Gln Glu Asp Ile Ala Arg Ile Ser
85 90 95

Lys Asp Met Glu Asp Ser Val Gly Leu Asp Cys Lys Arg Tyr Leu Gly
100 105 110

Leu Thr Val Ala Ser Phe Leu Gly Leu Leu Val Phe Leu Thr Pro Ile
115 120 125

Ala Phe Ile Leu Leu Pro Pro Ile Leu Trp Arg Asp Glu Leu Glu Pro
130 135 140

Cys Gly Thr Ile Cys Glu Gly Leu Phe Ile Ser Met Ala Phe Lys Leu
145 150 155 160

Leu Ile Leu Leu Ile Gly Thr Trp Ala Leu Phe Phe Arg Lys Arg Arg
165 170 175

Ala Asp Met Pro Arg Val Phe Val Phe Arg Ala Leu Leu Leu Val Leu
180 185 190

Ile Phe Leu Phe Val Val Ser Tyr Trp Leu Phe Tyr Gly Val Arg Ile
195 200 205

Leu Asp Ser Arg Asp Arg Asn Tyr Gln Gly Ile Val Gln Tyr Ala Val
210 215 220

Ser Leu Val Asp Ala Leu Leu Phe Ile His Tyr Leu Ala Ile Val Leu
225 230 235 240

Leu Glu Leu Arg Gln Leu Gln Pro Met Phe Thr Leu Gln Val Val Arg
245 250 255

Ser Thr Asp Gly Glu Ser Arg Phe Tyr Ser Leu Gly His Leu Ser Ile
260 265 270

Gln Arg Ala Ala Leu Val Val Leu Glu Asn Tyr Tyr Lys Asp Phe Thr
275 280 285

Ile Tyr Asn Pro Asn Leu Leu Thr Ala Ser Lys Phe Arg Ala Ala Lys
290 295 300

His Met Ala Gly Leu Lys Val Tyr Asn Val Asp Gly Pro Ser Asn Asn
305 310 315 320

Ala Thr Gly Gln Ser Arg Ala Met Ile Ala Ala Ala Ala Arg Arg Arg
325 330 335

Asp Ser Ser His Asn Glu Leu Tyr Tyr Glu Glu Ala Glu His Glu Arg
340 345 350

Arg Val Lys Lys Arg Lys Ala Arg Leu Val Val Ala Val Glu Glu Ala
355 360 365

Phe Ile His Ile Gln Arg Leu Gln Ala Glu Glu Gln Gln Lys Ala Pro
370 375 380

Gly Glu Val Met Asp Pro Arg Glu Ala Ala Gln Ala Ile Phe Pro Ser
385 390 395 400

Met Ala Arg Ala Leu Gln Lys Tyr Leu Arg Ile Thr Arg Gln Gln Asn
405 410 415

Tyr His Ser Met Glu Ser Ile Leu Gln His Leu Ala Phe Cys Ile Thr
420 425 430

Asn Gly Met Thr Pro Lys Ala Phe Leu Glu Arg Tyr Leu Ser Ala Gly
435 440 445

Pro Thr Leu Gln Tyr Asp Lys Asp Arg Trp Leu Ser Thr Gln Trp Arg
450 455 460

Leu Val Ser Asp Glu Ala Val Thr Asn Gly Leu Arg Asp Gly Ile Val
465 470 475 480

Phe Val Leu Lys Cys Leu Asp Phe Ser Leu Val Val Asn Val Lys Lys
485 490 495

Ile Pro Phe Ile Ile Leu Ser Glu Glu Phe Ile Asp Pro Lys Ser His
500 505 510

Lys Phe Val Leu Arg Leu Gln Ser Glu Thr Ser Val
515 520

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<211> 2336
<212> DNA
<213> Homo sapiens

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attttatttt attcagctta taatatgact cgatggagga aaatttgata agcatgagag 180

aagaccattc ttttcatgtt cgttacagaa tggaagcttc ttgcctagag ctggccttgg 240

aaggggaacg tctatgtaaa tcaggagact gccgcgctgg cgtgtcattc tttgaagctg 300

cagttcaagt tggaactgaa gacctaaaaa cacttagcgc tatttacagc cagttgggca 360

atgcttattt ctatttgcat gattatgcca aagcattaga atatcaccat catgatttaa 420

ccettgcaag gactattgga gaccagctgg gggaagcgaa agctagtggt aatctgggaa 480

acaccttaaa agttcttggg aattttgacg aagccatagt ttgttgtcag cgacacctag 540

atatttccag agagcttaat gacaaggtgg gagaagcaag agcactttac aatcttggga 600

atgtgtatca tgccaaaggg aaaagttttg gttgccctgg tccccaggat gtaggagaat 660

ttccagaaga agtgagagat gctctgcagg cagccgtgga tttttatgag gaaaacctat 720

cattagtgac tgctttgggt gaccgagcgg cacaaggacg tgcctttgga aatcttggaa 780

acacacatta cctccttggc aacttcaggg atgcagttat agctcatgag cagcgtctcc 840

ttattgcaaa agaatttgga gataaagcag ctgaaagaag agcatatagc aaccttggaa 900

atgcatatat atttcttggt gaatttgaaa ctgcctcgga atactacaag aagacactac 960

tgttggcccg acagcttaaa gaccgagctg tagaagcaca gtcttgttac agtcttggaa 1020

atacatatac tttacttcaa gactatgaaa aggccattga ttatcatctg aagcacttag 1080

caattgctca agagctgaat gatagaattg gtgaaggaag agcatgttgg agcttaggaa 1140

atgcatacac agcactagga aatcatgatc aagcaatgca ttttgctgaa aagcacttgg 1200

aaatttcaag agaggttggg gataaaagtg gtgaactaac agcacgactt aatctctcag 1260

accttcaaat ggttcttggt ctgagctaca gcacaaataa ctccataatg tctgaaaata 1320

ctgaaattga tagcagtttg aatggtgtac tccccaagtt gggacgccgg catagtatgg 1380

aaaatatgga acttatgaag ttaacaccag aaaaggtaca gaactggaac agtgaaattc 1440

ttgctaagca aaaacctctt attgccaaac cttctgcaaa gctactcttt gtcaacagac 1500

tgaaggggaa aaaatacaaa acgaattcct ccactaaagt tctccaagat gccagtaatt 1560

ctattgacca ccgaattcca aattctcaga ggaaaatcag tgcagatact attggagatg 1620

aagggttctt tgacttatta agccgatttc aaagcaatag gatggatgat cagagatgtt 1680

gcttacaaga aaagaactgc catacagctt caacaacaac ttcttccact ccccctaaaa 1740

tgatgctaaa aacatcatct gttcctgtgg tatcccccaa cacggatgag tttttagatc 1800

ttcttgccag ctcacagagt cgccgtctgg atgaccagag ggctagtttc agtaatttgc 1860

cagggcttcg tctaacacaa aacagccagt cggtacttag ccacctgatg actaatgaca 1920

acaaagaggc tgatgaagat ttctttgaca tccttgtaaa atgtcaagga tccagattag 1980

atgatcaaag atgtgctcca ccacctgcta ccacaaaggg tccgacagta ccagatgaag 2040

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tacaaagaga tcaaaacaga gacactgact ttgggctaaa ggacttttttg caaaataatg 2160

ctttgttgga gtttaaaaat tcagggaaaa aatcggcaga ccattagtta ctatggattt 2220

attttttttc ctttcaaaca cggtaaggaa acaatctatt actttttttcc ttaaaaggag 2280

aatttatagc actgtaatac agcttaaaat atttttagaa tgatgtaaat agttaa 2336

<210> 6
<211> 677
<212> PRT
<213> Homo sapiens

<400> 6

Met Arg Glu Asp His Ser Phe His Val Arg Tyr Arg Met Glu Ala Ser
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Cys Leu Glu Leu Ala Leu Glu Gly Glu Arg Leu Cys Lys Ser Gly Asp
20 25 30

Cys Arg Ala Gly Val Ser Phe Phe Glu Ala Ala Val Gln Val Gly Thr
35 40 45

Glu Asp Leu Lys Thr Leu Ser Ala Ile Tyr Ser Gln Leu Gly Asn Ala
50 55 60

Tyr Phe Tyr Leu His Asp Tyr Ala Lys Ala Leu Glu Tyr His His His
65 70 75 80

Asp Leu Thr Leu Ala Arg Thr Ile Gly Asp Gln Leu Gly Glu Ala Lys
85 90 95

Ala Ser Gly Asn Leu Gly Asn Thr Leu Lys Val Leu Gly Asn Phe Asp
100 105 110

Glu Ala Ile Val Cys Cys Gln Arg His Leu Asp Ile Ser Arg Glu Leu
115 120 125

Asn Asp Lys Val Gly Glu Ala Arg Ala Leu Tyr Asn Leu Gly Asn Val
130 135 140

Tyr His Ala Lys Gly Lys Ser Phe Gly Cys Pro Gly Pro Gln Asp Val
145 150 155 160

Gly Glu Phe Pro Glu Glu Val Arg Asp Ala Leu Gln Ala Ala Val Asp
165 170 175

Phe Tyr Glu Glu Asn Leu Ser Leu Val Thr Ala Leu Gly Asp Arg Ala
180 185 190

Ala Gln Gly Arg Ala Phe Gly Asn Leu Gly Asn Thr His Tyr Leu Leu
195 200 205

Gly Asn Phe Arg Asp Ala Val Ile Ala His Glu Gln Arg Leu Leu Ile
210 215 220

Ala Lys Glu Phe Gly Asp Lys Ala Ala Glu Arg Arg Ala Tyr Ser Asn
225 230 235 240

Leu Gly Asn Ala Tyr Ile Phe Leu Gly Glu Phe Glu Thr Ala Ser Glu
245 250 255

Tyr Tyr Lys Lys Thr Leu Leu Leu Ala Arg Gln Leu Lys Asp Arg Ala
260 265 270

Val Glu Ala Gln Ser Cys Tyr Ser Leu Gly Asn Thr Tyr Thr Leu Leu
275 280 285

Gln Asp Tyr Glu Lys Ala Ile Asp Tyr His Leu Lys His Leu Ala Ile
290 295 300

Ala Gln Glu Leu Asn Asp Arg Ile Gly Glu Gly Arg Ala Cys Trp Ser
305 310 315 320

Leu Gly Asn Ala Tyr Thr Ala Leu Gly Asn His Asp Gln Ala Met His
325 330 335

Phe Ala Glu Lys His Leu Glu Ile Ser Arg Glu Val Gly Asp Lys Ser
340 345 350

Gly Glu Leu Thr Ala Arg Leu Asn Leu Ser Asp Leu Gln Met Val Leu
355 360 365

Gly Leu Ser Tyr Ser Thr Asn Asn Ser Ile Met Ser Glu Asn Thr Glu
370 375 380

Ile Asp Ser Ser Leu Asn Gly Val Leu Pro Lys Leu Gly Arg Arg His
385 390 395 400

Ser Met Glu Asn Met Glu Leu Met Lys Leu Thr Pro Glu Lys Val Gln
405 410 415

Asn Trp Asn Ser Glu Ile Leu Ala Lys Gln Lys Pro Leu Ile Ala Lys
420 425 430

Pro Ser Ala Lys Leu Leu Phe Val Asn Arg Leu Lys Gly Lys Lys Tyr
435 440 445

Lys Thr Asn Ser Ser Thr Lys Val Leu Gln Asp Ala Ser Asn Ser Ile
450 455 460

Asp His Arg Ile Pro Asn Ser Gln Arg Lys Ile Ser Ala Asp Thr Ile
465 470 475 480

Gly Asp Glu Gly Phe Phe Asp Leu Leu Ser Arg Phe Gln Ser Asn Arg
485 490 495

Met Asp Asp Gln Arg Cys Cys Leu Gln Glu Lys Asn Cys His Thr Ala
500 505 510

Ser Thr Thr Thr Ser Ser Thr Pro Pro Lys Met Met Leu Lys Thr Ser
515 520 525

Ser Val Pro Val Val Ser Pro Asn Thr Asp Glu Phe Leu Asp Leu Leu
530 535 540

Ala Ser Ser Gln Ser Arg Arg Leu Asp Asp Gln Arg Ala Ser Phe Ser
545 550 555 560

Asn Leu Pro Gly Leu Arg Leu Thr Gln Asn Ser Gln Ser Val Leu Ser
565 570 575

His Leu Met Thr Asn Asp Asn Lys Glu Ala Asp Glu Asp Phe Phe Asp
580 585 590

Ile Leu Val Lys Cys Gln Gly Ser Arg Leu Asp Asp Gln Arg Cys Ala
595 600 605

Pro Pro Pro Ala Thr Thr Lys Gly Pro Thr Val Pro Asp Glu Asp Phe
610 615 620

Phe Ser Leu Ile Leu Arg Ser Gln Gly Lys Arg Met Asp Glu Gln Arg
625 630 635 640

Val Leu Leu Gln Arg Asp Gln Asn Arg Asp Thr Asp Phe Gly Leu Lys
645 650 655

Asp Phe Leu Gln Asn Asn Ala Leu Leu Glu Phe Lys Asn Ser Gly Lys
660 665 670

Lys Ser Ala Asp His
675

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<211> 22
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

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acaacagcct caagatcatc ag 22

<210> 8
<211> 20

<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 8
ggtccaccac tgacacgttg 20

<210> 9
<211> 22
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 9
agctgagaca tttgttctct tg 22

<210> 10
<211> 22
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 10
tataaaccag ctgagtccag ag 22

<210> 11
<211> 22

<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 11
ctcacttggc acgtcagcag gg 22

<210> 12
<211> 10
<212> DNA
<213> Homo sapiens

<400> 12
cccgccatgc 10

<210> 13
<211> 16
<212> DNA
<213> Artificial

<220>
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<400> 13
tgctccggca tggcgg 16

<210> 14
<211> 16
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized oligonucleotide sequence

<400> 14
gctgaactgc tccggc 16

<210> 15
<211> 16
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized oligonucleotide sequence

<400> 15
tccaagatct cctccc 16

<210> 16
<211> 16
<212> DNA
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<223> an artificially synthesized oligonucleotide sequence

<400> 16
tctccttcca agatct 16

<210> 17
<211> 16
<212> DNA
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<223> an artificially synthesized oligonucleotide sequence

<400> 17

gcgctgagcc ggcctc 16

<210> 18

<211> 16

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized oligonucleotide sequence

<400> 18

cctcacctcc tcccgc 16

<210> 19

<211> 16

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized oligonucleotide sequence

<400> 19

ccgccatgcc ggagca 16

<210> 20

<211> 16

<212> DNA

<213> Artificial

<220>
<223> an artificially synthesized oligonucleotide sequence

<400> 20
gccggagcag ttcagc 16

<210> 21
<211> 16
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized oligonucleotide sequence

<400> 21
gggaggagat cttgga 16

<210> 22
<211> 16
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized oligonucleotide sequence

<400> 22
agatcttgga aggaga 16

<210> 23
<211> 16
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized oligonucleotide sequence

<400> 23
gaggccggct cagcgc 16

<210> 24
<211> 16
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized oligonucleotide sequence

<400> 24
gcgggaggag gtgagg 16

<210> 25
<211> 23
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 25
gagttgtatt atgaagaggc cga 23

<210> 26
<211> 23
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 26
atgtctcaga ctgtaagcga agg 23

<210> 27
<211> 28
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 27
tgtcagctct ccgcttgcgg aaaaaaag 28

<210> 28
<211> 16
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<220>
<223> an artificially synthesized oligonucleotide sequence

<400> 28
gtatccatag caatgg 16

<210> 29
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(74) Agents: **SHIMIZU, Hatsushi** et al.; Kantetsu Tsukuba Bldg. 6F, 1-1-1, Oroshi-machi, Tsuchiura-shi, Ibaraki 300-0847 (JP).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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Published:
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(88) Date of publication of the international search report: 24 December 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/027322 A3

(54) Title: HEPATOCELLULAR CARCINOMA-RELATED GENES AND POLYPEPTIDES, AND METHOD FOR DETECTING HEPATOCELLULAR CARCINOMAS

(57) Abstract: Genes up-regulated in hepatocellular carcinomas and polypeptides encoded by these genes are provided. Vectors, transformants and methods for producing the recombinant polypeptides are also provided. Probes and primers of these genes and antibodies against the polypeptides are also provided. The probes, primers and antibodies can be used as reagents for detecting hepatocellular carcinomas. Methods for detecting hepatocellular carcinomas using such detection reagents are further provided. Antisense nucleotide sequences of these genes are also provided and can be used to inhibit growth of hepatocellular carcinomas.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 02/09873

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 17355 A (INCYTE PHARMA INC ;PATTERSON CHANDRA (US); AZIMZAI YALDA (US); COR) 30 March 2000 (2000-03-30) page 41, line 15 -page 44, line 18 page 53, line 5 - line 34 page 56, line 22 -page 57, line 2 claims 1,9,12-14,16 in particular: SEQ ID Nos. 3 and 19 ---	1-9
X	DATABASE EMBL 'Online! EBI22 February 2000 (2000-02-22) SUGANO S.: "Homo sapiens cDNA FLJ20199 fis" retrieved from HTTP://WWW.EBI.AC.UK Database accession no. AK000206 XP002235526 the whole document --- -/--	1,3-5,8

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 2003	01.07.03

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ulbrecht, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 02/09873

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! EBI16 May 2001 (2001-05-16) NCI-MGC: "602661486F1 NIH_MCG_21 Homo sapiens cDNA clone IMAGE:4810124 5'" retrieved from HTTP://WWW.EBI.AC.UK Database accession no. BG773806 XP002235527 the whole document ---	1,3-5,8
A	YING HAO ET AL: "Cloning and characterization of F-LANa, upregulated in human liver cancer." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 286, no. 2, 17 August 2001 (2001-08-17), pages 394-400, XP002235523 ISSN: 0006-291X abstract page 396, right-hand column, paragraph 1 figures 1,2 ---	1-10
A	VON MARSCHALL Z ET AL: "Dual mechanism of vascular endothelial growth factor upregulation by hypoxia in human hepatocellular carcinoma." GUT, vol. 48, no. 1, January 2001 (2001-01), pages 87-96, XP009008136 ISSN: 0017-5749 abstract page 91, right-hand column, paragraph 3 -page 93, right-hand column, paragraph 2 table 2 figures 1-3 ---	1-10
A	OKABE HIROSHI ET AL: "Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: Identification of genes involved in viral carcinogenesis and tumor progression." CANCER RESEARCH, vol. 61, no. 5, 1 March 2001 (2001-03-01), pages 2129-2137, XP002235524 ISSN: 0008-5472 table 1 ---	1-10

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 02/09873

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	TANAKA KENJI ET AL: "Enhanced expression of mRNAs of antisecretory factor-1, gp96, DAD1 and CDC34 in human hepatocellular carcinomas." BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1536, no. 1, 2001, pages 1-12, XP002235525 ISSN: 0006-3002 abstract page 4, left-hand column, paragraph 2 -page 5, left-hand column, paragraph 2 page 8, left-hand column, paragraph 2 -right-hand column, paragraph 2 figures 1,2,4 ---	1-10
A	WO 99 39200 A (UNIV JEFFERSON ;FEITELSON MARK A (US)) 5 August 1999 (1999-08-05) page 4, line 10 -page 6, line 2 example 4 page 13, line 10 -page 17, line 30 claim 1 ---	1-10
A	JACKSON T R ET AL: "Cytohesins and centaurins: mediators of PI 3-kinase-regulated Arf signaling" TIBS TRENDS IN BIOCHEMICAL SCIENCES, ELSEVIER PUBLICATION, CAMBRIDGE, EN, vol. 25, no. 10, 1 October 2000 (2000-10-01), pages 489-495, XP004224289 ISSN: 0968-0004 page 490, right-hand column, paragraph 2 -page 491, left-hand column, paragraph 2 -----	1,2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 02/09873

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 7, 10
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery
Although claims 7 and 10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the parts of

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 02/09873

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

the said method that do not relate to surgery or treatment performed on the human/animal body.

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10 (all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 7 and 10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the parts of the said method that do not relate to surgery or treatment performed on the human/animal body.

Continuation of Box I.1

Claims Nos.: 7, 10

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 02/09873

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-10 (all partially)

Invention 1:

An isolated nucleic acid selected form the group consisting of: a nucleic acid comprising SEQ ID No. 1, a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID No. 2, a nucleic acid comprising a strand that hybridizes to a nucleic acid consisting of SEQ ID No. 1; a vector carrying the said nucleic acid; a transformant carrying the said vector or nucleic acid; a method of producing a polypeptide using the said transformant; an isolated polypeptide selected form the group consisting of: a polypeptide encoded by the nucleic acid sequence of SEQ ID No. 1, a polypeptide comprising the amino acid sequence of SEQ ID No. 2, a polypeptide having at least 65% identity to SEQ ID No. 2; an antibody that specifically binds to the said polypeptide; a method for detecting hepatocellular carcinoma (HCC) comprising measuring the expression level of the polypeptide of SEQ ID No. 1; a reagent for detecting HCC which hybridises to a nucleotide sequence of SEQ ID No. 1; a reagent for detecting HCC comprising the said antibody; a method for inhibiting growth of HCC using an antisense oligonucleotide which hybridises with the nucleotide sequence of SEQ ID No. 1.

2. Claims: 1-10 (all partially)

Invention 2:

An isolated nucleic acid selected form the group consisting of: a nucleic acid comprising SEQ ID No. 3, a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID No. 4, a nucleic acid comprising a strand that hybridizes to a nucleic acid consisting of SEQ ID No. 3; a vector carrying the said nucleic acid; a transformant carrying the said vector or nucleic acid; a method of producing a polypeptide using the said transformant; an isolated polypeptide selected form the group consisting of: a polypeptide encoded by the nucleic acid sequence of SEQ ID No. 3, a polypeptide comprising the amino acid sequence of SEQ ID No. 4, a polypeptide having at least 65% identity to SEQ ID No. 4; an antibody that specifically binds to the said polypeptide; a method for detecting hepatocellular carcinoma (HCC) comprising measuring the expression level of the polypeptide of SEQ ID No. 3; a reagent for detecting HCC which hybridises to a nucleotide sequence of SEQ ID No. 3; a reagent for detecting HCC comprising the said antibody; a method for inhibiting growth of HCC using an antisense oligonucleotide which hybridises with the nucleotide sequence of SEQ ID No. 3.

International application No.
PCT/JP 02/09873

3. Claims: 7, 8, 10 (all partially)

Invetion 3:

A method for detecting hepatocellular carcinoma (HCC) comprising measuring the expression level of the polypeptide of SEQ ID No. 5; a reagent for detecting HCC which hybridises to a nucleotide sequence of SEQ ID No. 5; a method for inhibiting growth of HCC using an antisense oligonucleotide which hybridises with the nucleotide sequence of SEQ ID No. 5.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/JP 02/09873

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0017355 A	30-03-2000	CA 2343360 A	30-03-2000
		EP 1114158 A	11-07-2001
		JP 2002526076 T	20-08-2002
		AU 6048799 A	10-04-2000
WO 9939200 A	05-08-1999	AU 2480799 A	16-08-1999